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SYNTHETIC ANALOGS OF PHOSPHOLIPID METABOLITES AS ANTIMALARIALS.(U)
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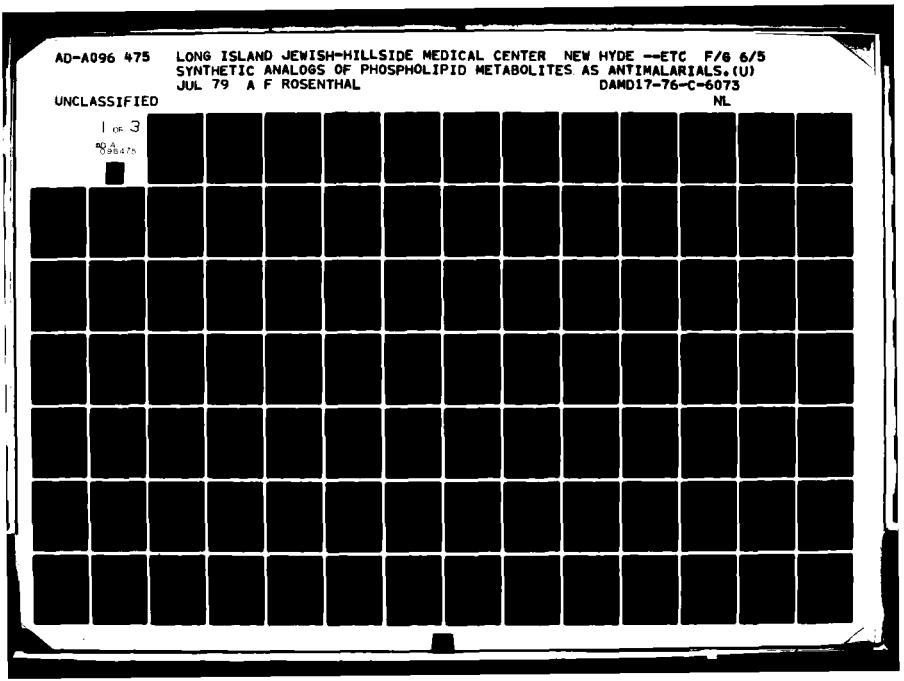

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Phospholipid analogs. Phospholipid biosynthetic intermediate analogs. CDP-diglyceride analogs. CDP-choline (-ethanolamine) analogs. Lipid antimalarials. Phosphonates. Phosphinates. Non-hydrolyzable lipid analogs. Ether phospholipids. Organophosphorus synthetic chemistry.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Analogs of phospholipids and their biosynthetic intermediates were synthetic targets as novel potential antimalarials. Targeted analogs contained one or more types of non-hydrolyzable groups, including ether, phosphonate and phosphinate moieties. Synthetic routes varied with each target, and were quite complex for the cytidine-containing analogs of the biosynthetic intermediates, involving a number of novel steps.
The phospholipid analogs were successfully prepared and submitted.

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20. (continued)

However, during the course of the contract syntheses of the major cytidine-containing target compounds could not be completed, although a monophosphonate "model analog" was prepared and submitted.

None of the thirteen compounds (including three targets and one "model target") submitted displayed appreciable antimalarial activity *in vivo* in the standard test system.

AD _____

SYNTHETIC ANALOGS OF PHOSPHOLIPID METABOLITES AS ANTIMALARIALS

FINAL TECHNICAL REPORT

By

Arthur F. Rosenthal, Ph.D.

July 1979

(for the period 1 July 1976 - 30 September 1978)

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-76-C-6073

Long Island Jewish-Hillside Medical Center
New Hyde Park, N.Y. 11042

BRIEF SUMMARY

The synthesis of a number of analogs of phospholipids and their biosynthetic intermediates was undertaken with a view toward preparing novel antimalarial substances which acted against plasmodial lipid metabolism. The target compounds included phosphatidic acid analogs containing ether and phosphonate groups; completely non-hydrolyzable lecithin analogs containing phosphinate and ether groups; a lecithin analog containing abnormal base and ether groups; and cytidine diphosphate-diglyceride (CDPDG) analogs containing one, two and three methylene groups in place of the phosphorus ester and pyrophosphate moieties. The corresponding analogs of cytidine diphosphate choline (CDPC) and cytidine diphosphate ethanolamine (CDPE) were more distant targets whose syntheses would utilize much of the methodology developed in the synthesis of the CDPDG analogs.

The synthetic methods employed toward each target compound differed greatly from one group to another and are given in the text. The routes to the CDPDG (and the corresponding CDPC and CDPE) analogs particularly were quite complex and involved at many individual steps new areas of organophosphorus chemistry for which current knowledge was either sketchy or non-existent. These steps thus proved more time-consuming than might have appeared to be the case. The details of the synthetic routes are too lengthy to be clearly summarized and are given schematically in figures in the text.

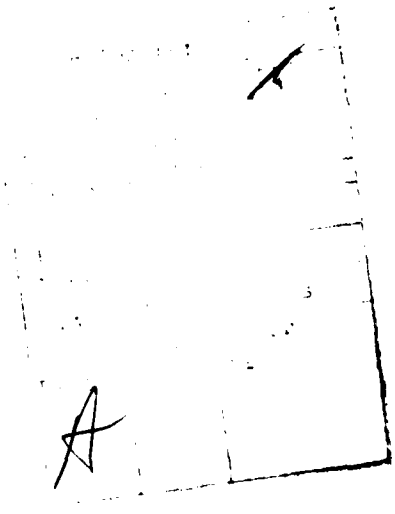
The phosphatidic acid and lecithin target compounds were successfully synthesized and submitted, together with a number of intermediates. A model of a CDP-diglyceride analog containing only one phosphorus moiety (as phosphonate) was synthesized and submitted. Syntheses of no major CDPDG, CDPC or CDPE target analogs could be completed within the time allotted for the contract research, but the synthetic routes progressed to a significant extent, particularly for the CDPDG analogs containing two methylene groups, replacing the diglyceride phosphorus ester and pyrophosphate groups. Several intermediates from synthetic routes to various analogs of the CDP-containing biosynthetic intermediates were submitted for testing.

A total of thirteen compounds were submitted, of which three were targets, one a "model target", and nine were intermediates. None of the compounds submitted showed appreciable antimalarial (or in one case, anti-Leishmaniasis) activity.

From the work done during the course of this contract it could only be concluded that lecithin analogs, including one which had previously been shown to inhibit venom phospholipase A, fail to prevent the reproduction of Plasmodia in vivo. This could be due to lack of intracellular penetration by the analogs, to differences in the characteristics of the Plasmodial phospholipases, or to competitive fixation of the analogs to other cell or plasma constituents in the host. It might also signify that phospholipid hydrolysis by Plasmodia is not an essential source of fatty acid for parasite growth, in contradistinction to prior literature.

No conclusions could be drawn about the effects of analogs of CDP-containing biosynthetic intermediates, since only a monophosphonate-containing analog was tested during the contract period.

TABLE OF CONTENTS



1. Introduction.

In this final report emphasis of detail is placed upon that portion of the work which was completed or in progress since the last quarterly report (March 31, 1978). For a discussion of the details of work done prior to that date the reader is referred to the previous seven reports, including the 1977 Annual Report.

1.1. General Aims.

The work to be described has as its primary aim the synthesis of analogs of certain phospholipid metabolites and biosynthetic intermediates which could have selective deleterious effects upon Plasmodia, and thus act as useful antimalarial agents. Very few of such analogs affecting lipid metabolism have been examined previously as possible antimalarials, and certain features of Plasmodial metabolism made this approach appear as a novel and perhaps promising approach to malarial chemotherapy.

The compounds chosen as targets were designed to (a) inhibit the availability of fatty acids to the Plasmodial cells, which were believed to require these metabolites for their biosynthesis of phospholipids; or (b) inhibit the formation, hopefully on a selective basis, of the phospholipids by providing metabolic inhibitors of the biosynthetic intermediates.

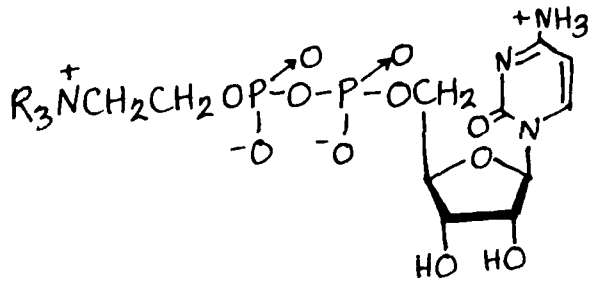
1.2. Rationale.

In the field of antimalarial therapy it is unusual for a specific area of parasite metabolism to be the target of antimetabolite therapy a priori; i.e., with few if any

antimalarials of the type in question to serve as precedents. In the case of Plasmodial phospholipid metabolism such an approach is rendered still more unusual by the paucity of known specific inhibitors of phospholipid metabolism, particularly biosynthesis, in any microbial or mammalian system.

The primary justification for such an approach lies in the apparently critical importance of phospholipid biogenesis to plasmodial maturation, at least intra-erythrocytically, the site in which its development has been most extensively studied. Moreover, since the steps of phospholipid biosynthesis are well known, there exist a number of rational points at which to attempt to produce metabolic antagonism by the use of analogs of the pertinent metabolites.

It has been known for some time that Plasmodia, particularly in the well-studied intraerythrocytic stages, have a very active lipid metabolism compared to their host erythrocytes(1-3). In recent years this has been shown to be particularly true of their phospholipid biogenesis, of which many details have been clarified. The transferase reactions



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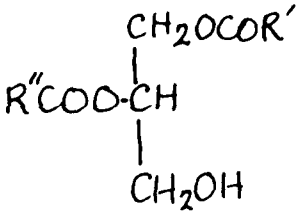
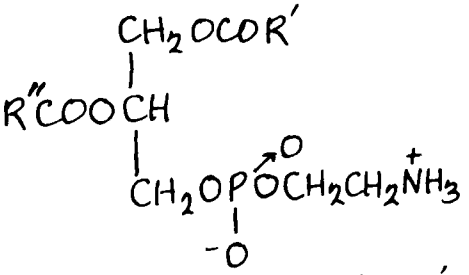


Transferase →



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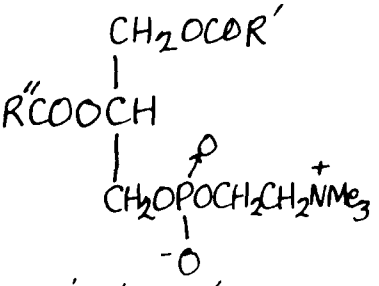
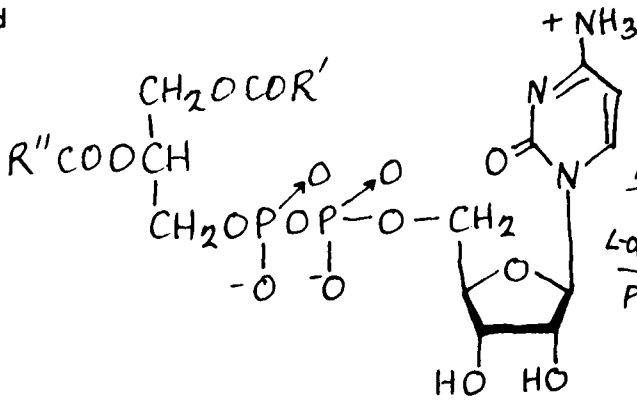


Phosphatidylethanolamine (PE)

Phosphatidylcholine (Lecithin, PC)

R = H or Me R′ and R″ = Long-chain alkyl

and



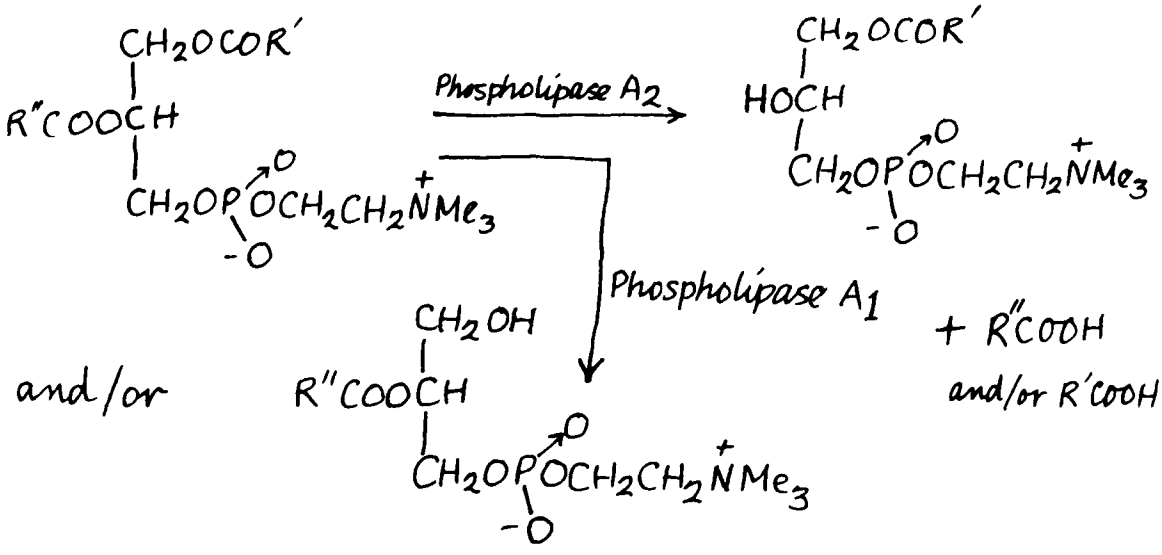
L-Serine → Phosphatidylserine (PS)

Myoinositol → Phosphatidylinositol (PI)

L-α-Glycerophosphate, Phosphatase, etc. → Phosphatidylglycerol (PG), etc.

are apparently both highly active, since ethanolamine and choline are readily incorporated into Plasmodial membrane PE and PC (2), and lipids produced by the latter pathway, such as PI and PG, are readily formed from labelled phosphate (3). Phospholipids formed by both pathways are the major lipid components of Plasmodial membranes (4, 5).

A possibly vulnerable but in any event highly significant point of Plasmodial lipid metabolism is the inability of the organisms to synthesize fatty acids de novo; thus the source of fatty acids for this active phospholipid metabolism is the host erythrocyte and plasma lipids (2, 3, 6). There exists no clearer example of chemical parasitism by these organisms. The erythrocyte membrane phospholipids are hydrolyzed rapidly by a phospholipase A:



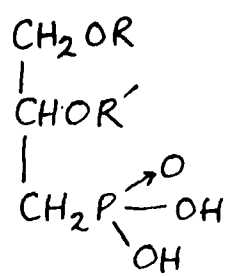
This reaction and similar reactions with plasma phospholipid, as well as plasma free fatty acids, probably supplies the major portion of the fatty acid requirements for Plasmodial growth and development.

2. Target Compounds.

It must be borne in mind that the lipid analogs containing long chain groups can exist as families of compounds differing in the chain length and degree of unsaturation of the long chain groups, like the natural lipid metabolites themselves. The choice of R groups is thus somewhat arbitrary and is made on the basis of resemblance to the natural compounds and for convenience in working out the synthetic methods which should then be applicable to other homologues. In addition, compounds containing base moieties can be made of either choline or ethanolamine-containing substances, both of which are found in the natural metabolites.

2.1. Phosphatidic Acid Analogs.

Phosphatidic acids occupy a key role in both the biosynthesis of glycerides and the formation of phospholipids. Diether phosphonate analogs of the following type

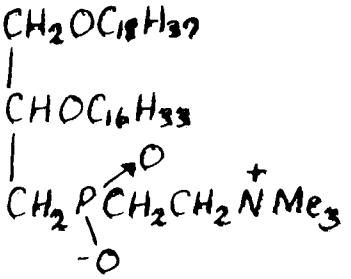


I

have been already shown (7, 8) to be inhibitory towards phosphatidic acid phosphatase, a key enzyme in αβ - diglyceride formation.

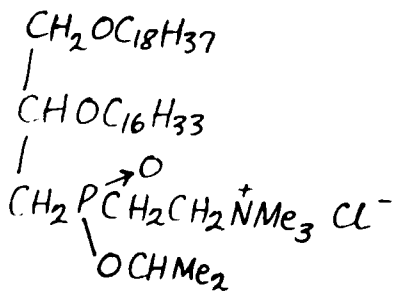
2.2. Lecithin Analogs.

Insofar as phospholipases can be inferred to be necessary for the phospholipid turnover of Plasmodial membranes, it seems reasonable to prepare a lecithin analog which has previously been shown to exert appreciable antiphospholipase A (venom enzyme) and also antiphospholipase C (clostridial enzyme) activity (9, 10) This substance is a completely non-hydrolyzable analog of lecithin containing ether and phosphonate moieties instead of the normally labile carboxylic and phosphoric acid groups.



II

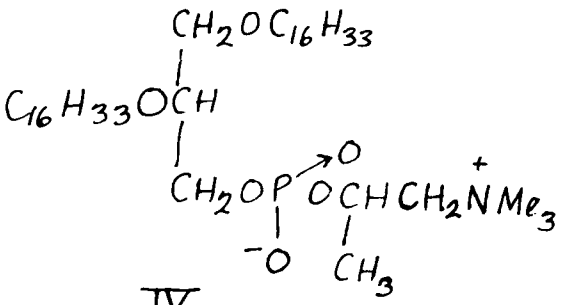
In addition, its immediate synthetic precursor, the isopropyl ester chloride salt



III

was taken as a secondary target compound. Its lesser ionic charge was believed to offer some additional hope of increased intracellular penetrability.

A lecithin analog of rather different type was also a target compound. Although no biochemical information was available on this compound, it is somewhat more similar to a natural lecithin than the phosphinate analog described above. It differs primarily in having an unusual, branched-chain base in place in choline.

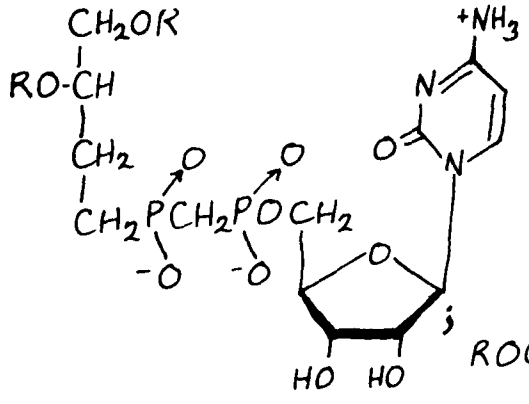


IV

2.3. Analogs of Cytidine Diphosphate — Diglyceride

Cytidine diphosphate diglycerides are a family of unique liponucleotides which are obligatory intermediates in the biosynthesis of phosphatidylserine, with possible decarboxylation to phosphatidylethanolamine, of phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and several other less common phospholipids. (The only exception to the previous statement is the fact that in some organisms deoxycytidine diphosphate diglyceride can substitute for the corribose liponucleotides; the requirement for cytidine moiety is essentially absolute, however.)

The liponucleotide analogs which are the ultimate targets of these synthetic efforts are the following:



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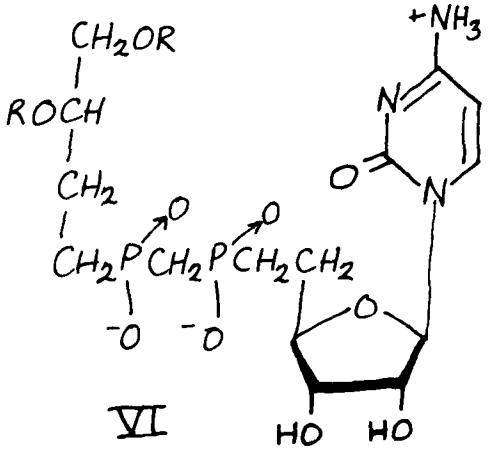
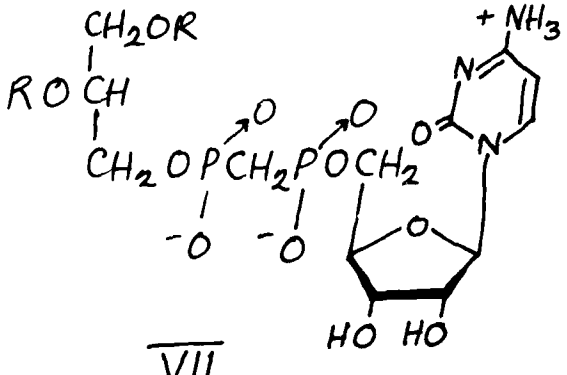


VI

In addition, an intense but circumscribed effort was made to prepare a methylenediphosphonic acid analog of the following structure:

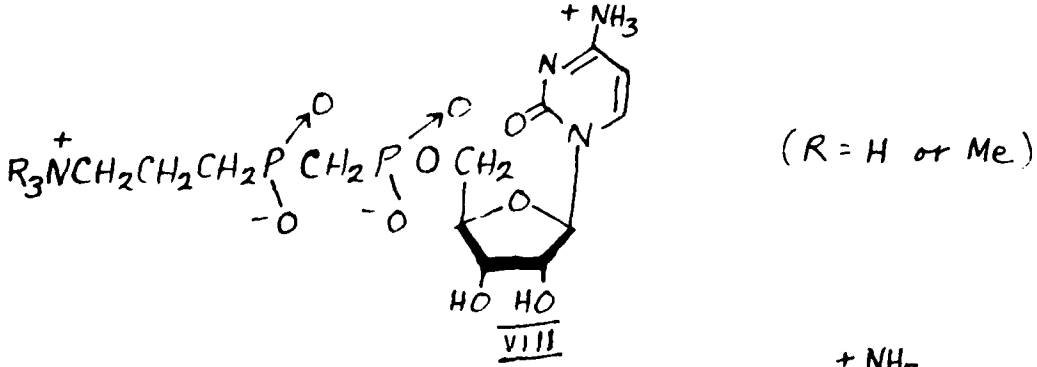


VII

2.4. Analogs of Cytidine Diphosphate Choline and Cytidine Diphosphate Ethanolamine.

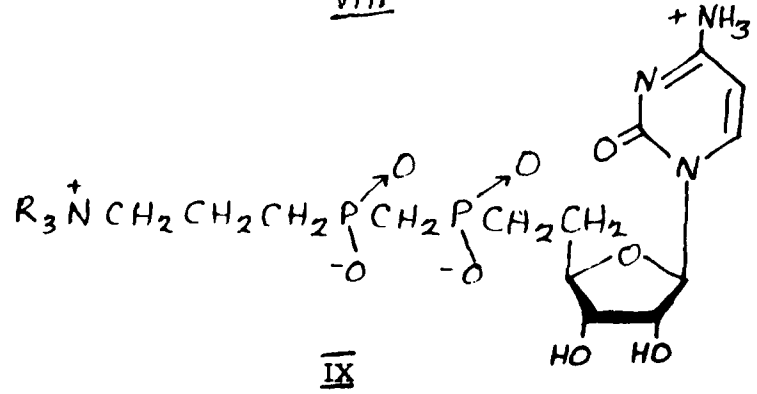
The non-liponucleotide coenzymes cytidine diphosphate choline and -ethanolamine utilize 1,2-diglyceride rather than phosphatidic acid as the co-reactant, to produce lecithin and phosphatidyl ethanolamine directly. The coenzyme thus acts as a phosphorylated base donor rather than as a phosphatidyl donor, as in the case of CDP-diglyceride. This route is probably the major one for the formation of lecithin at least, and interference with the reaction should exert a significant effect on membrane phospholipid formation.

The target analogs of the base-dinucleotides have the same relationship to their natural coenzymes as do the liponucleotide analogs discussed above, and have the following structure:



(R = H or Me)

VIII



IX

3. Overview of Results.

The contract was funded from July 1, 1976, until September 30, 1978. The full personnel complement requested was made available to the Laboratory between November, 1977 and the expiration of the contract.

During the total period of tenure of the contract several target compounds of simpler structure, together with several secondary target compounds were submitted. In addition, a number of intermediates which had been purified to an analytical level were also submitted. No active compounds were found in the standard antimalarial test or in the Leishmanniasis test. Most of the key target compounds, particularly of the cytidine nucleotide metabolic analog type, were still incomplete at the conclusion of the contract. These latter types of compounds, of course, occupied most of the effort expended during the contract period, at least after the first four months from the inception. (During this initial period almost all the effort, as requested by WRAIR, was put into the resynthesis of a large amount of one of the lecithin analogs for which a synthetic procedure had been already worked out.)

The "research" portion of the project (as distinct from the "production" portion) was characterized primarily by efforts to work out adequate synthetic methods for preparing the cytidine intermediates discussed briefly in section 2. This effort was characterized by the starts, stops, retrenchments, and sometimes fundamental alterations in synthetic route common to most synthetic efforts, but greatly magnified by the fact that at key points along the route critical information

on the behavior of organophosphorus compounds was sparse or lacking.

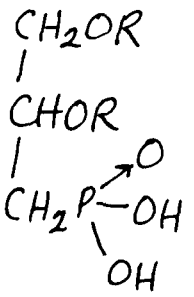
However, in addition to the need for alterations occasioned by purely synthetic problems some changes in procedure were occasioned for happier reasons, such as the appearance of new literature which appeared to make possible simplifications; or to new methods or reagents which were prepared in the course of our own work.

Thus, the results showed that with the manpower resources available to us the contractual period was not sufficient to complete the critical syntheses undertaken. However, enough progress was made so that at least some of the key cytidine-containing liponucleotide and base analogs might be expected to be prepared within a reasonable additional period of time.

4. Specific Procedures and Results.

4.1. Phosphatidic Acid Analogs.

The synthetic scheme for compounds of type I is shown in figure 1.



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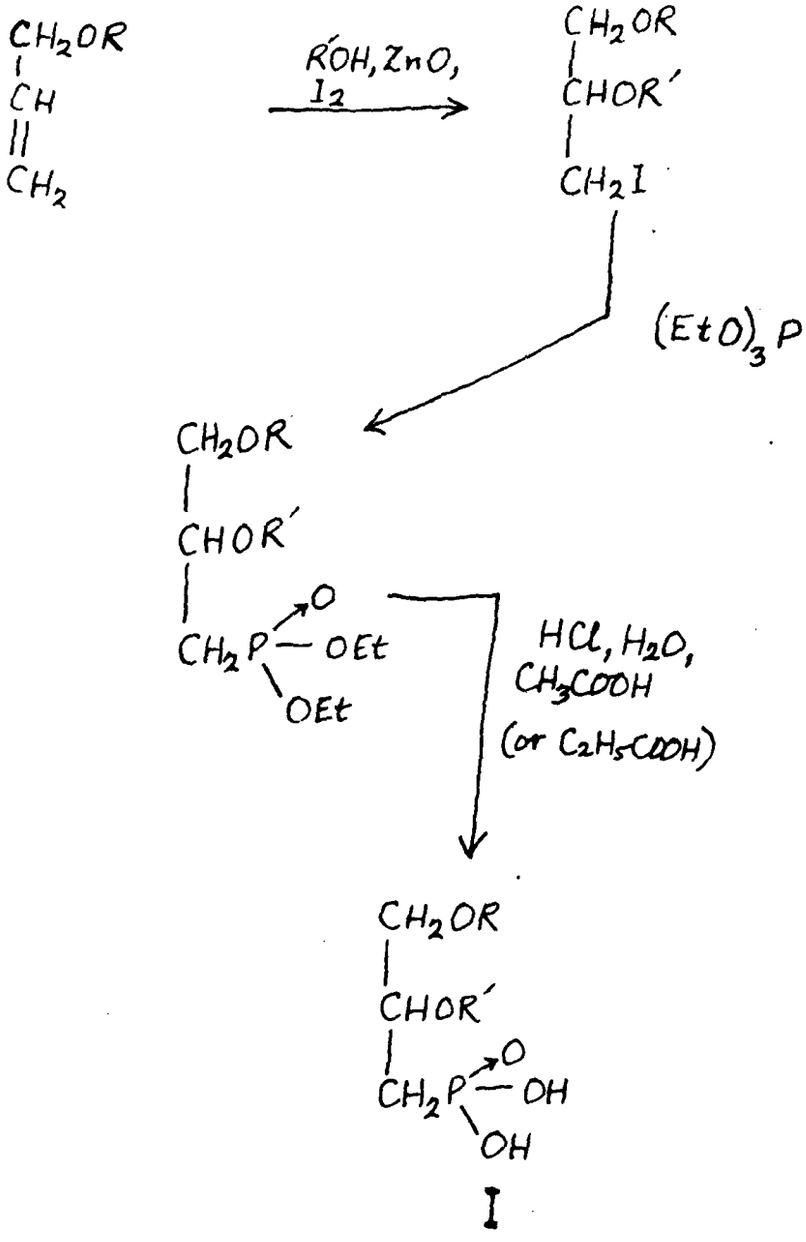

CH2OR
CH
CH2
R'OH, ZnO,
I2
CH2OR
CHOR'
CH2I
(EtO)3P
CH2OR
CHOR'
CH2P
O
OEt
OEt
HCl, H2O,
CH3COOH
(or C2H5COOH)
CH2OR
CHOR'
CH2P
O
OH
OH

I

FIG 1

The synthetic steps have already been extensively discussed in previous reports[a,b]. The dihexadecyl homologue of I ($R=C_{16}H_{33}$) was prepared and submitted for testing. It did not show appreciable antimalarial activity. The intermediate 2, 3-dihexadecyl-oxyiodopropane was also submitted and also proved to be without antimalarial activity, but was surprisingly non-toxic.

4.2. Lecithin Analogs.

The synthesis of the lecithin analog II was taken as the initial project in the contract period by request of WRAIR. The synthetic route is shown in figure 2.

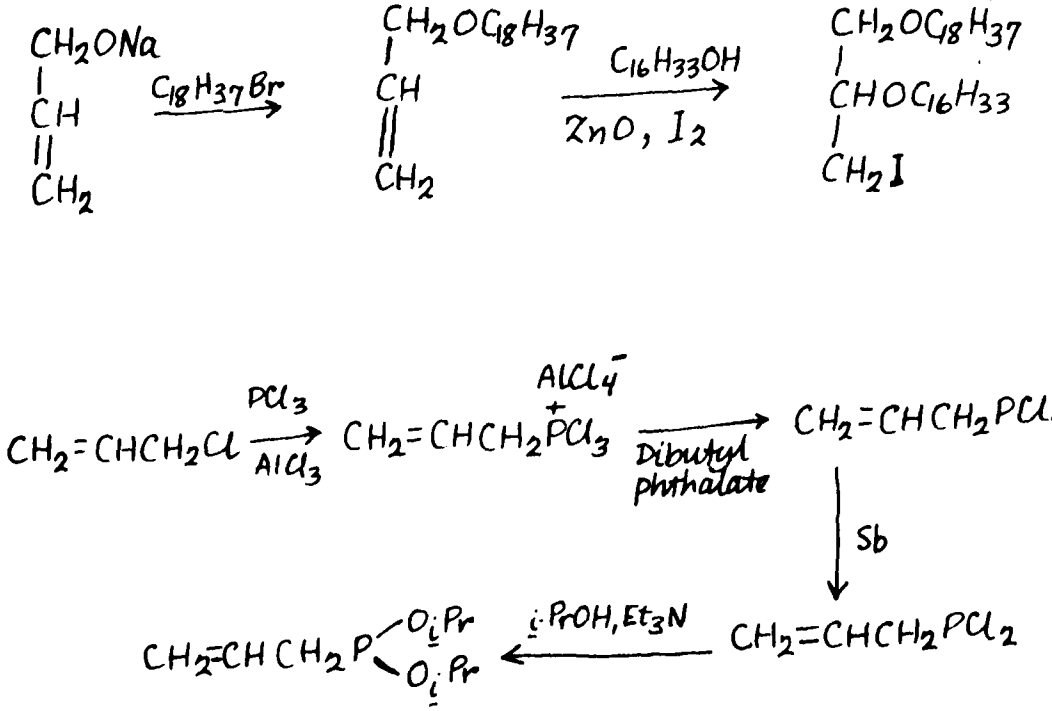


Fig 2

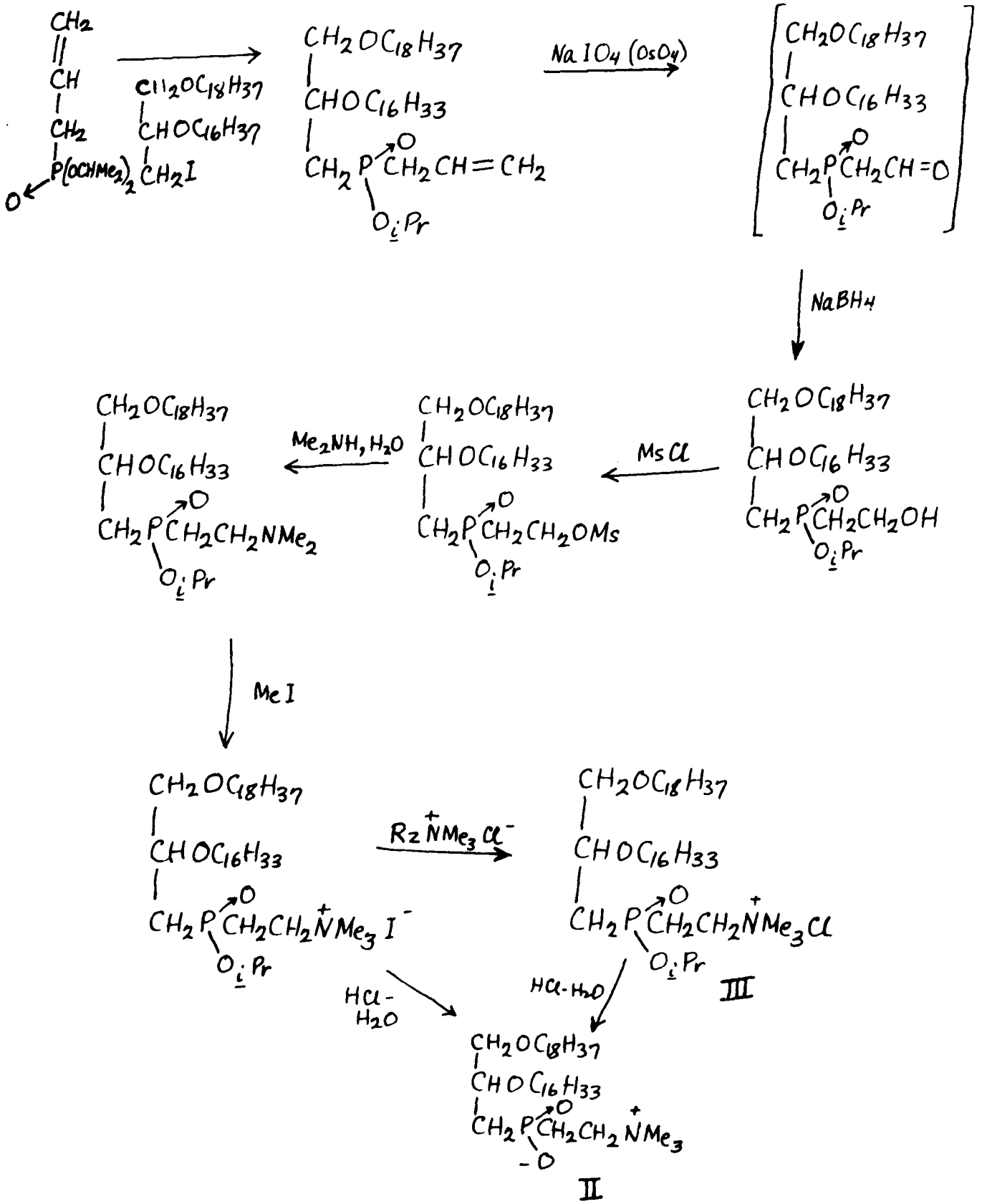


Fig 2 (continued)

The details of the synthesis have already been discussed extensively [a,b]. The isopropyl ester chloride salt III was also prepared in sufficient quantity for submission and testing [c]. Neither the lecithin analog nor its ester showed appreciable antimalarial or anti-Leishmanniasis activity. Both, however, were very non-toxic.

The lecithin analog IV was also prepared [b]; it too failed to show significant antimalarial activity but was rather non-toxic to the experimental animals.

4.3. Cytidine Diphosphate Diglyceride Analogs.

During the period of the total contract the greatest portion of the efforts of laboratory personnel was directed toward this series of compounds. Not only were these compounds primary targets in themselves, but in many respects they served in addition as model compounds for the corresponding cytidine diphosphate choline analogs. As will be seen below, many of the intermediates which constitute the key compounds leading to the CDP - diglyceride analogs are also usable for formation of the CDP - choline analogs. In general, emphasis was placed initially on the former class of substances, since the long chain saturated groups usually produce intermediates which are readily crystalized. Although this does not usually produce an initially very pure compound, at least the solubility properties of all the intermediates are more predictable than in the case of the CDP -base analogs, and can serve to explore many of the synthetic steps common to both classes of compounds.

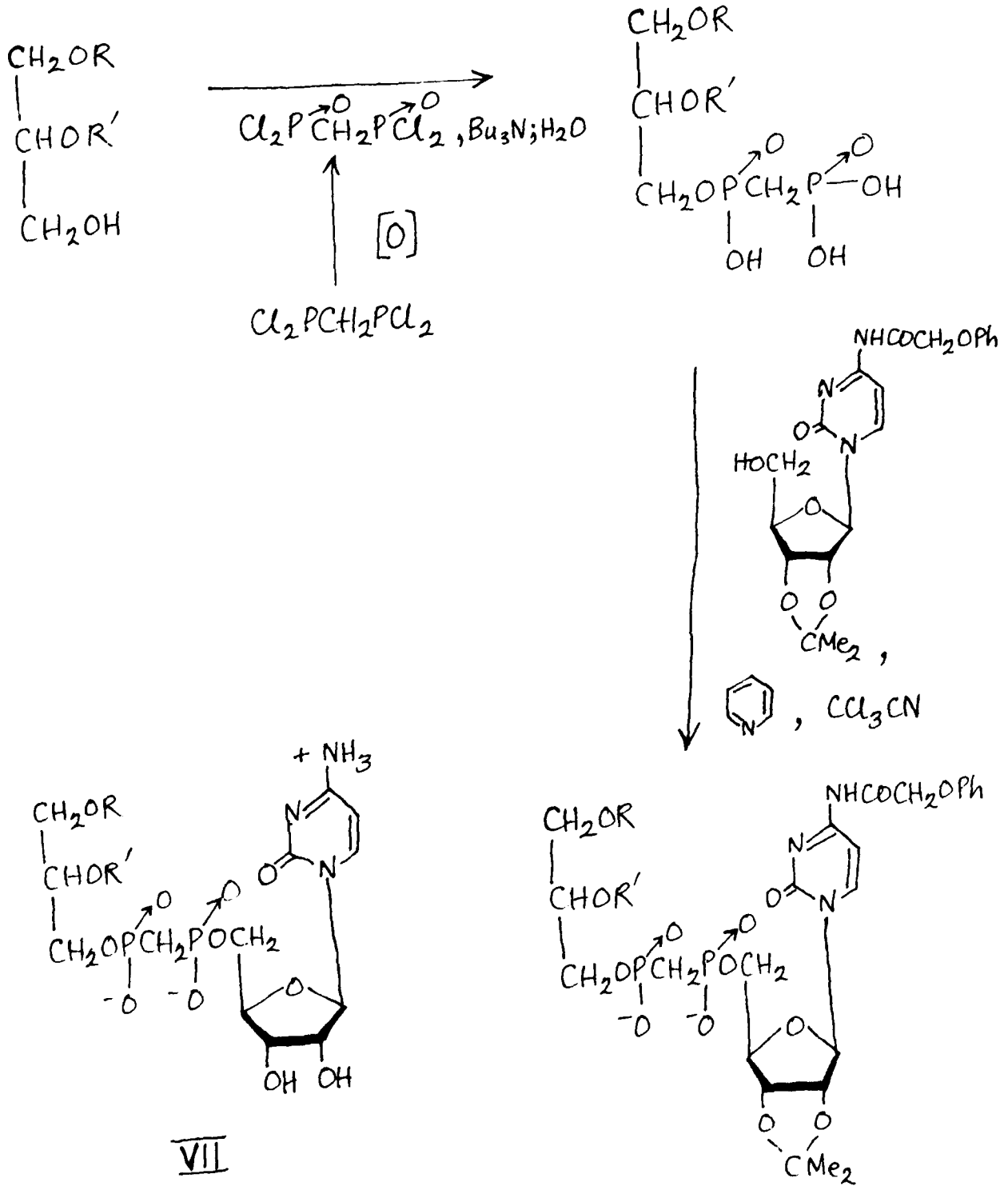
4.3.1. Analogs Containing The -O-P-CH_2-P-O- Group.

A rather brief but intensive effort was made to synthesize a compound of this type by using the route shown in figure 3. It can be seen that this effort involves a simple and well known type of phosphorus acid condensation with the diglyceride analog, but employs a rather problematical condensation with the cytidine derivative in which the unused phosphorus functions are unprotected. The justification for this approach lies in the relative ease with which methylene-diphosphonic acid is obtained relative to its partial esters of known position, which would be necessary for protection of the appropriate acid functions.

The preparation of the glycerol diether starting material has of course been reported in the literature many times, but the synthetic procedures are somewhat tedious. In order to take advantage of the availability of a significant amount of the iodo-diether

$$\begin{array}{l} CH_2OC_{18}H_{37} \\ | \\ CHOC_{16}H_{33} \\ | \\ CH_2I \end{array},$$

used in the preparation of the lecithin analog II, a study of the transformation of this iodide into the glycerol diether was first made. It was recognized at the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CH2OH
Cl2PCH2PCl2 , Bu3N;H2O
[O]
Cl2PCH2PCl2
CH2OR
CHOR'
CH2OPCH2POH
OH OH
NHCOCH2OPh
HOCH2
CMe2 ,
, CCl3CN
+ NH3
CH2OR
CHOR'
CH2OPCH2POCH2
-O -O
OH OH
VII
NHCOCH2OPh
CH2OR
CHOR'
CH2OPCH2POCH2
-O -O
CMe2

Fig 3

outset that β-halo ethers are considerably less reactive than ordinary corresponding alkyl halides, a property only somewhat obviated by the fact that the more reactive iodo group was present. It could not therefore be expected that the transformation of this iodide to an alcoholic moiety would proceed in very high yield, but the ease of preparation and availability of the iodo compound nevertheless made this approach seem far more attractive than a de novo synthesis.

Reaction of the iodo-diether with sodium benzoxide was slow and not complete. Reaction of the iodo-diether in a water-methyl isobutyl ketone phase-transfer reaction proceeded but at an even slower rate. However, reaction of sodium benzoate with the iodo compound in refluxing dimethylformamide proceeded fairly satisfactorily and gave the expected glycerol diether benzoate. Presumably, the higher temperature obtainable with this solvent made the reaction proceed adequately; this combination has been reported to be highly nucleophilic (11, 12).
In any event the benzoate was saponified to the alcohol, accompanied by a chromatographically removable but otherwise rather persistent impurity. Reduction of the optically active glyceraldehyde diether (see below) and comparison of the resulting optically active glycerol diether with the racemic compound obtained from the iodo compound showed that they were otherwise identical. Thus, migrations and other rearrangements can be excluded and, although only a moderate yield of diether was obtained, the method can be highly recommended for the production of significant quantities of this intermediate.

In any event this was only a prelude to the main problem, which was, of course, to obtain a selective esterification in each of two steps, the first being the reaction of methylenediphosphonic tetrachloride with only one molecule of the glycerol diether, and the second the esterification condensation promoted by trichloroacetonitrile or a similar condensing agent.

First, however, the tetrachloride itself had to be prepared. The procedures given in the literature are quite tedious and in fact some of the intermediates are no longer commercially available. With the availability of methylenediphosphonous tetrachloride (see below), however, it seemed that this substance could provide an attractive alternative starting material for the preparation of the phosphonic tetrachloride. A large number of oxidation reactions were run, using both sulfuryl chloride and dry air as the oxidants. Best results were obtained by bubbling a stream of dry air through neat methylenediphosphonous tetrachloride for several hours.

Reaction of a large excess of methylenediphosphonic tetrachloride and pyridine with glycerol 1,2-dioctadecyl ether yielded an acidic product which was difficult to purify. However, on analysis the product was found to contain approximately half the amount of phosphorus of that expected, and to show a much higher molecular weight than anticipated. The only reasonable interpretation of these results is that two, rather than only one, molecules of the alcohol had reacted with the tetrachloride to produce a bis-ester of unknown (probably mixed) ester position, despite the large excess of tetrachloride used. In fact, for the period this reaction was under investigation conditions could not be found in which this

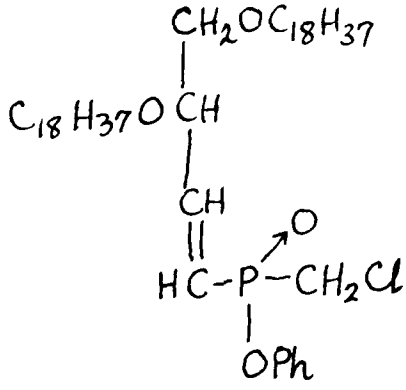
problem could be avoided and bis products were always found as the major ones. Thus, no further work could be accomplished on the synthesis until this problem could be overcome.

Obviously, various more complex synthetic routes could also be investigated, but it seems probable that these would take a much larger amount of time and effort than the simple three-step procedure above. Thus, with the time and resources available it was necessary to shelve the synthesis of these analogs for the time being.

4.3.2. Analogs Containing The $-CH_2P\langle CH_2P\langle O$ - Group.

Since only one phosphorus ester group is present in analogs of type IV it is not surprising that a very different route was contemplated for the first three quarters of the synthesis compared to that for the previous type of compound. The synthetic scheme is shown in figure 4. The initial requirement was the synthesis of a large amount of glyceraldehyde diether, which, as mentioned above, had already been synthesized some time previously. A significant improvement, much more convenient for large scale reactions, was made by employing for the first time a phase-transfer reaction for the synthesis of a long chain ether to produce the mannitol hexaether fairly conveniently as compared with the earlier reaction with powdered potassium hydroxide in dioxane. This modification was published (13), and has been previously discussed in detail [a].

The complete synthetic route proposed for synthesis of IV is given in figures 4 and 5. The unsaturated chloromethyl phosphinic ester intermediate



which requires five steps to prepare, also provided a series of synthetic difficulties, some of which were not obvious a priori. Actually this compound had been prepared some time before the inception of the contract, but on a small scale, so that considerable resynthesis was necessary. Rigorous efforts to purify this intermediate did not produce a successful result on a sufficient scale. Column chromatography was the method which had been studied most extensively; while some pure material could be obtained thereby, most of the material appeared to be irreversibly adsorbed to the column. This problem has already been discussed in considerable detail [c]. In subsequent work, therefore, it was necessary to use the crude Wittig products as the starting material for subsequent steps and to attempt to purify later intermediates which might prove more amenable to chromatographic separation.

Another problem associated with this chloromethyl intermediate is that it contains an activated halogen but a double bound which is deactivated to hydrogenation but activated to addition reactions. Thus, although in an isolated run it proved

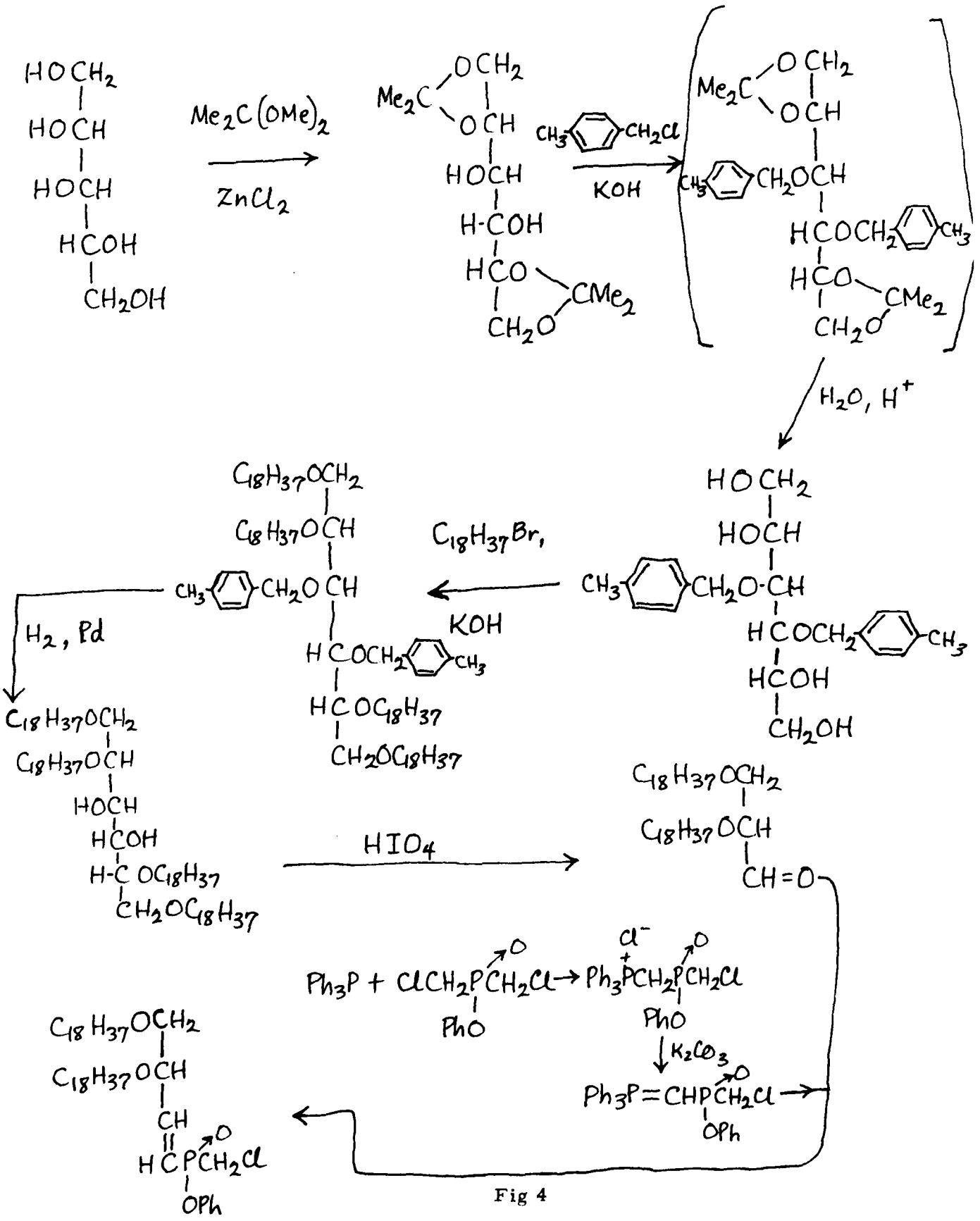

HOCH2
HOCH
HOCH
HCOH
CH2OH
Me2C(OMe)2
ZnCl2
OCH2
Me2C
OCH
HOCH
H-COH
HCO
CMe2
CH2O
CH3
CH2Cl
KOH
CH2OCH
HCOCH2
H2O, H+
C18H37OCH2
C18H37OCH
C18H37Br,
KOH
H2, Pd
HCOC18H37
CH2OC18H37
HCOH
CH2OH
HIO4
CH=O
Ph3P + ClCH2PCH2Cl
PhO
Cl-
Ph3PCH2PCH2Cl
K2CO3
Ph3P=CHPCH2Cl
OPh
HCPCH2Cl

Fig 4

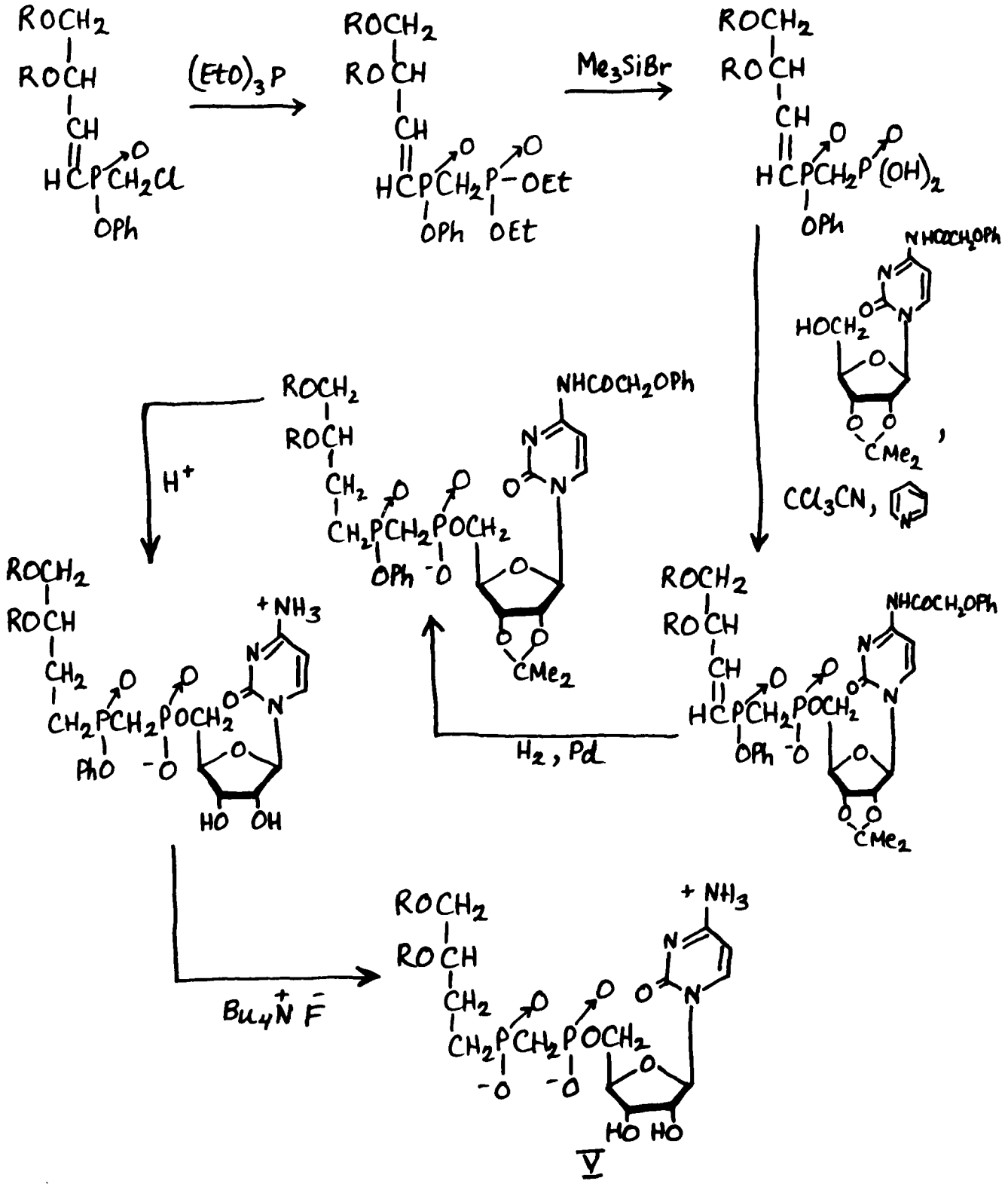

ROCH2
ROCH
CH
HCP
O
CH2Cl
OPh
(EtO)3P
CH2P
OEt
Me3SiBr
(OH)2
NHCOCH2OPh
HOCH2
CMe2
CCl3CN,
H+
NHCOCH2OPh
CH2PCH2POCH2
OPh -O
H2, Pd
+NH3
PhO
HO OH
Bu4N+ F-
V

Fig 5

possible occasionally to prepare a pure saturated chloromethyl compound by reduction with palladium and hydrogen, usually this reaction could not be controlled to stop at that stage and chlorine-free products were often obtained. Presumably this irreproducibility was due to minor variations in the activity of the catalysts but in any event resulted in a decision to employ the unsaturated chloromethyl compound in the Arbuzov reaction, and then to reduce the product, and finally to attempt its purification.

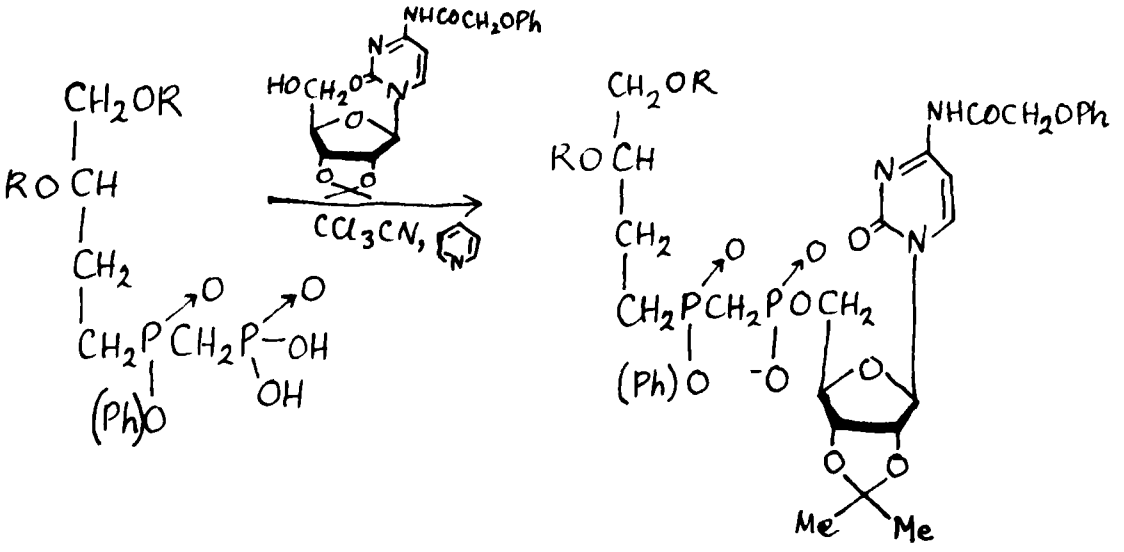
An additional somewhat unexpected difficulty was that the formation of the Arbuzov products is occompanied by a very significant amount of dephenylation in the course of its reaction with tris (trimethylsilyl) phosphite. The amount of dephenylation proved as difficult to control as the hydrogenation of the chloromethyl group in the previous intermediate; it usually ranged from about half to perhaps 80-90%.

This unexpected reaction was studied considerable detail. It was first of all conjectured that the active dephenylating agent might be the by-product trimethylsilyl chloride, in which case the reaction could be greatly improved by putting a relatively inert competing phenyl ester into the reaction mixture. Addition of triphenylphosphite or triphenylphosphate to the Arbuzov reaction mixture, however, had no effect on the dephenylation. When the volatile by-products of the reaction were trapped through a condenser, aromatic compounds could actually be found in the condensate, whether or not these competing phenyl esters were present. It must thus be concluded that the dephenylation reaction is actually a neighboring

group effect, probably proceeding through a cyclic intermediate, and can thus not be significantly influenced by added substrates.

Moreover, the dephenylation is produced by the trimethylsilyl group only, since conventional Arbuzov reagents do not produce this unwanted side reaction. Some time ago it was found that an analytically pure diethyl phosphonate-phosphinate with its phenyl phosphinate ester group intact could be produced by reaction with triethyl phosphite. At the time, this appeared to be a dead-end route because adequate methods were not at the time available for selective removal of the ethyl groups, however. In any event it proved possible by long and tedious purification to isolate from the reaction with tris (trimethylsilyl) phosphite a pure phenyl - containing phosphinate phosphonic acid in low yield[e], but it was obvious that this was not the main product and that attempting this preparation in an unaltered form would prove inefficient in the use of difficultly obtainable intermediates.

On reflection it seems far from certain that the condensation - esterification step



would in fact require that the phosphinic acid function be protected by esterification. It may also be possible that the ionic forms of the phosphinic acid and the primary phosphonic acid groups would actually be anionic in pyridine, and that this would act as a "protecting" group for the reaction of the much weaker and presumably mainly unionized second hydrogen of the phosphonic acid group. It may be recalled that a similar projection was made for the simple route to the methylenediphosphonic acid analogs at the second esterification step, although the route did not progress far enough to actually test this possibility (see above).

In order to avoid the formation of a mixture of products and to control the steps at which removal of protecting groups could be accomplished, the crude chloromethyl phosphinic ester was reacted with triethyl phosphite and the product purified and hydrogenated. The resulting product was difficult to characterize because the unsaturated and saturated compounds have essentially identical Rf's, even on silver nitrate - impregnated silica gel (presumably due to a number of somewhat bulky groups in the vicinity of the double bond). Spectral data which was available to us was also inconclusive, since only one internal double bond in a large molecule was present or absent.

At any rate the product was reacted with trimethylsilyl bromide in an attempt to selectively remove the aliphatic ester groups. Although investigation of model compounds such as diphenyl chloromethylphosphonate indicated that in such substances the phenyl groups are left intact by reaction with trimethylsilyl bromide, this was not the case with the phosphinate-phosphonate triester. Here the

phenyl group was removed as readily as the ethyls and no conditions could be found under which only the aliphatic esters are removed. Apparently, we were again dealing with some kind of neighboring group effect probably mediated through a cyclic intermediate or transition state.

The deprotected phosphinic - phosphonic acid was not at this stage extensively purified, because chromatographic methods gave rather diffuse spots or zones of adsorption common to acids of this type. Furthermore, it was of course the object at this stage to discover whether or not the condensation with the protected cytidine would proceed in pyridine solution without protection of the phosphorus functions. Thus, a large number of such reactions were studied, both by analytical TLC and on a preparative scale. Chronologically this work was done close to the end of the contract period, and by its expiration purification of the products formed was in progress. Generally two products were formed from this condensation - esterification reaction, both of which were ultraviolet - absorbing, and phosphorus - containing. They differed in Rf from the model compound, one having a lower and the other a higher Rf than the latter.

Extensive earlier work with the products formed in the tris (trimethylsilyl) phosphite reaction are important and pertinent as well. While this work was in progress, however, it became necessary to obtain as a reference, as well as a proof that the condensation reaction really would work even with an isolated phosphonic acid group, to obtain a model cytidine ester. Additionally, if such a compound were formed it could serve as a model for the final step, namely the deprotection of the product of esterification to give the final cytidine liponucleotide

analog.

For this purpose 2-hexadecoxy-3-octadecoxypropylphosphonic acid was reacted with N-phenoxyacetyl-2', 3' - isopropylidene-cytidine in pyridine solution. The expected cytidine derivative was obtained in good yield. Deprotection required more vigorous conditions than anticipated (aqueous trifluoroacetic acid at 45° for a number of hours) but gave the expected cytidine liponucleotide model in very good yield. Details of this synthesis have already been reported[d]; the compound was submitted for antimalarial testing but proved inactive. This is possibly to be expected since it does not contain the putatively requisite -P-O-P- or -P-C-P- groups.

Studies of the condensation - esterification using acids obtained by Arbuzov reaction with tris (trimethylsilyl) phosphite have always been hampered by the several products obtained in the Arbuzov reaction and the subsequent acqueous hydrolysis. As indicated above, acids of this nature are very difficult to purify chromatographically, since they diffusely adsorb to silica in a variety of solvents. Only in strongly acidic solvents (e.g. trifluoroacetic acid in alcohol-free chloroform) do such compounds form compact spots, but such solvent mixtures are very difficult to handle on a preparative basis, may produce some unexpected side reactions, and in any event do not produce very much variation in the migration of the desired products away from closely related impurities. Thus, a long and tedious separation of diffusely adsorbed acid products was resorted to and it was combined fractions from these separations which were used as the starting acid in the condensation reactions. After the condensation reaction was complete with

each of the various fractions, deprotection of the products was carried out and separation of the various compounds thus produced was undertaken by both column chromatography and thick-layer chromatography. This effort occupied a very large amount of our time during the project period, and some of this work has already been reported [b, c]. The results, however, can be summarized by reporting that the compounds produced by this method ultimately proved unsatisfactory in purity and yield, and the phenyl diethyl phosphinic ester- phosphonic ester route discussed above was undertaken in its stead with the expectation of greater success. As indicated above, This effort was not yet complete at the expiration of the contract.

4.3.4. Analogs Containing the $-CH_2\overset{/}{P}\backslash CH_2\overset{/}{P}\backslash CH_2-$ Group.

These analogs of type VIare in many respects the most interesting of all the cytidine diphosphate diglyceride analogs. All obvious points of hydrolytic lability have been eliminated by a formal substitution with a methylene moiety, save for the unusually stable glycosidic linkage between the ribose and cytidine groups. Such substances might be expected to retain their biological activity for an extended period of time, limited primarily by excretory rather than metabolic processes.

However, the presence of two unsymmetrical phosphinic acid groups in the molecule, for which relatively few synthetic procedures are available, makes the synthesis of these compounds potentially more difficult than any of those above.

The first route considered is in reality an extension of that projected for

compounds of type V, making use of the reagent $Me_3SiCH_2P(OSi\ Me_3)_3$ rather than tris (trimethylsilyl) phosphite as in the previous compounds. This reagent was prepared some time ago (14) and shown to be an effective Arbuzov reagent for the formation of unsymmetrical phosphinic acids containing the hydroxymethyl group (Fig 6).

Before we considered this portion of the synthesis, however, it was evident to us that any route which we might employ for the synthesis of compounds of type VI would require a Wittig reaction to be carried out on a 5'-aldehyde of a protected cytidine. Since the oxidation of cytidine derivatives to 5'-aldehydes had not been reported in the literature although those containing other base groups had been known for several years, it was necessary to discover whether or not there was anything particular about cytidine derivatives which made them unsuitable for this type of oxidation reaction. Therefore, the preparation of this type of cytidine derivative was first investigated. Reaction of N-phenoxyacetyl-2', 3'-isopropylidinecytidine with dimethylsulfoxide and dicyclohexylcarbodiimide in the presence of dichloroacetic acid gave what appeared on thin layer chromatography to be a virtually quantitative yield of the expected aldehyde. Isolation of this compound required, however, the formation of a more stable derivative; for this purpose the dianilinoethane adducts seemed most useful. The adduct, however, did not form under conditions employed for other nucleotide derivatives but formed very readily when a trace of acetic acid was added to the reaction mixture. The product was isolated in good yield and a quantity sent to WRAIR for antimalarial testing. It proved to be inactive and non-toxic.

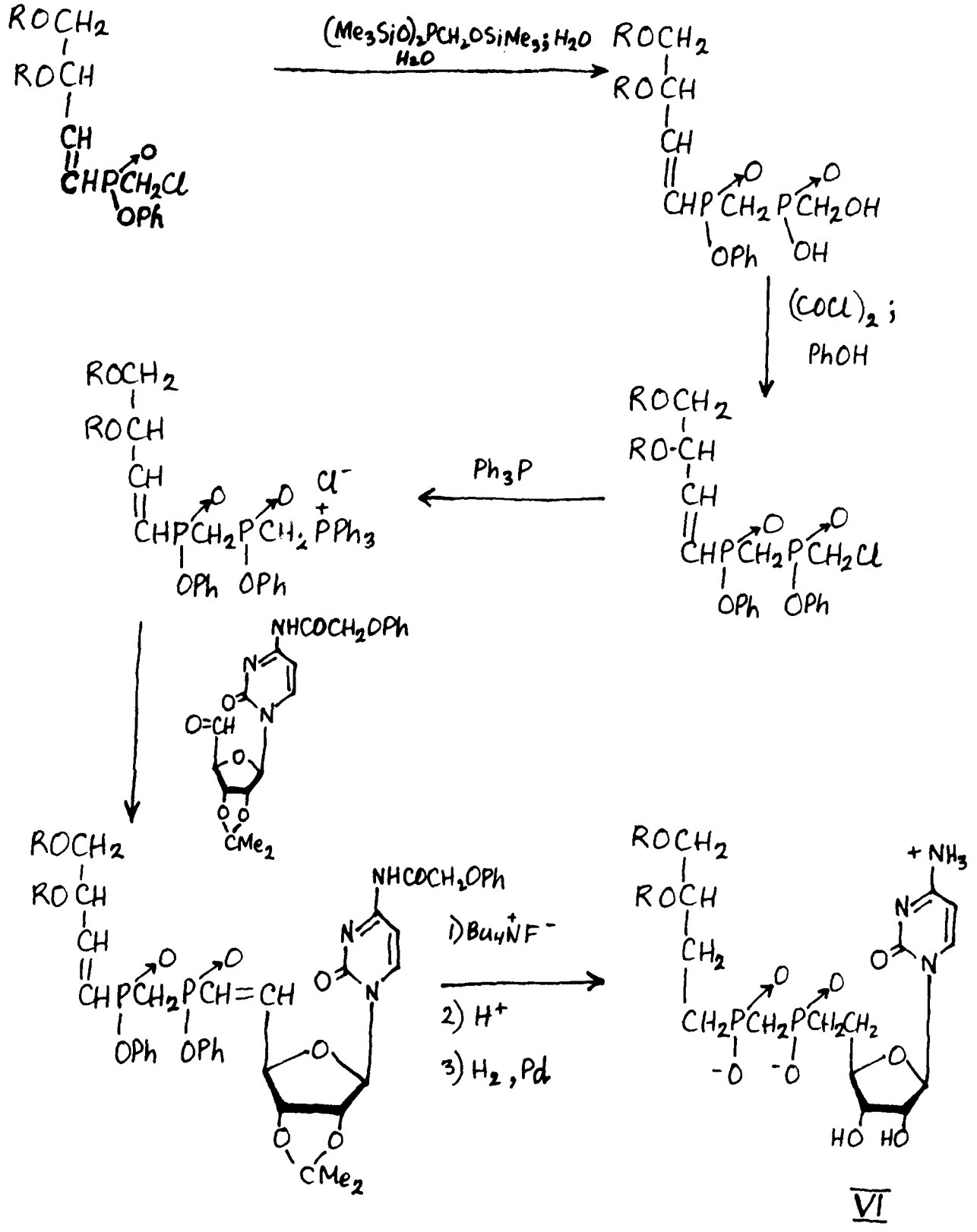
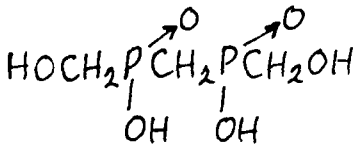

(Me3SiO)2PCH2OSiMe3; H2O
H2O
(COCl)2;
PhOH
Ph3P
NHCOCH2OPh
CMe2
1) Bu4N+F-
2) H+
3) H2, Pd
+NH3
VI

Fig 6

This potentially valuable and versatile intermediate is made by reaction of methylene chloride with aluminum, and subsequent reaction of the bis (chloromethyl) aluminum chloride formed with phosphorus trichloride. A large amount of this intermediate was in fact prepared by via by this elegant method without much difficulty, as has already been reported [f, g].

Obviously the success of this synthetic route would depend upon the transformation of this tetrachloride into subsequent intermediates such as bis (hydroxymethyl) methylene diphosphinic acid,



and for this reason additional work was concentrated on this portion of the molecule, assuming that introduction of the lipid portion of the molecule will proceed more or less as in the case of prior compounds once a suitable Wittig reagent is available.

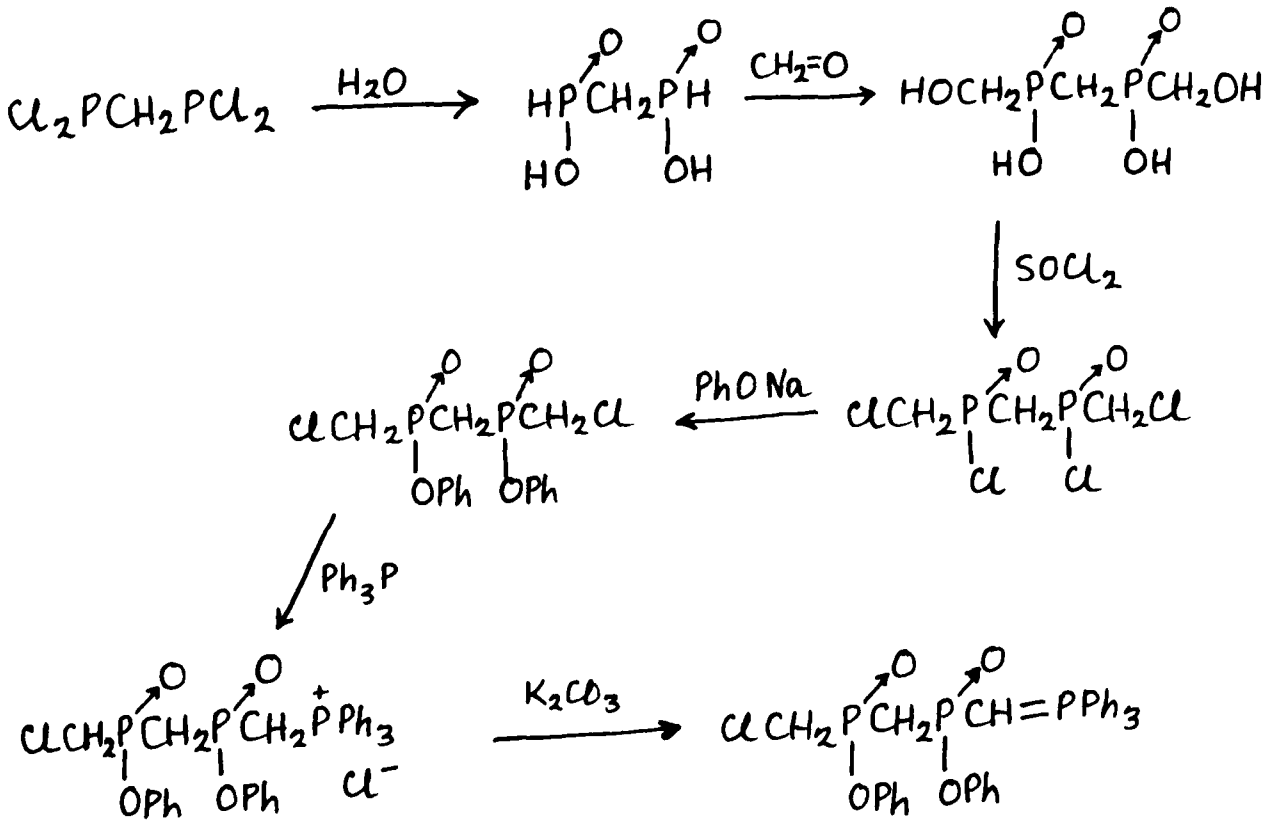
Careful hydrolysis of the tetrachloride to the bis-phosphonous acid was accomplished at low temperature, using concentrated hydrochloric acid as the hydrolytic agent to minimize localized heat release caused by the heat of solution of hydrogen chloride in water. After thorough removal of hydrochloric acid the residue was rendered as anhydrous as possible by azeotropic distillation with isopropyl alcohol and the product was obtained as a viscous oil which gave a satisfactory n m r spectrum. An alternative side-route in which hydrolysis was accomplished in

Mild acidic hydrolytic conditions could not be found under which the dianilinoethane adduct could be reconverted to N-phenoxyacetyl-2',3'-isopropylidenecytidine-5'-aldehyde without significant removal of other protecting groups. Thus, it was concluded that for synthetic purposes the aldehyde must be prepared just before use without forming any derivatives. For reasons that may be evident by looking at the activated methylene group straddled by two phosphorus functions in the final projected molecule, the use of base-removable protecting groups was avoided. This work has previously been reported in detail [9].

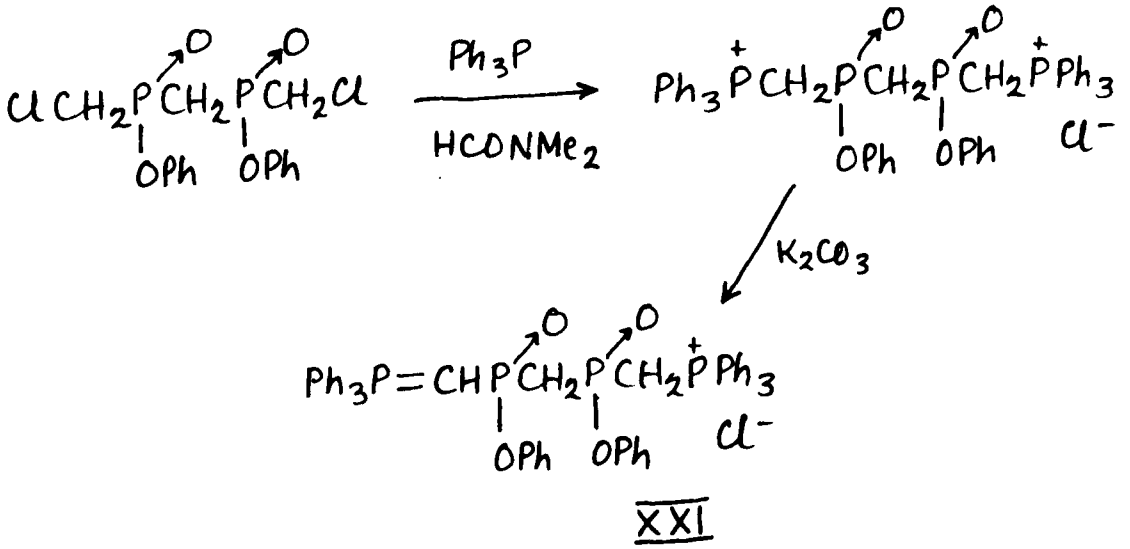
This synthetic route had an interesting history inasmuch as it was initially the object of a good deal of effort centering around the Arbuzov reaction, but was for the time being held in suspension while what seemed a more promising route was investigated. Earlier work on this reaction has already been reported [b, e]. Repetition of the reaction and purification of the compound produced gave a product which was fairly promising by elemental analysis. Not enough compound was available for detailed examination and additional purification. It seems likely that with the acquisition of additional information relative to the conversion of such a compound into its diester halomethyl derivative the use of this route would become much more attractive.

The second route to analogs of type VI became available with a report in the literature of a simple means of making methylenediphosphonous tetrachloride,

$Cl_2PCH_2PCl_2$, (Figs 7 and 8)



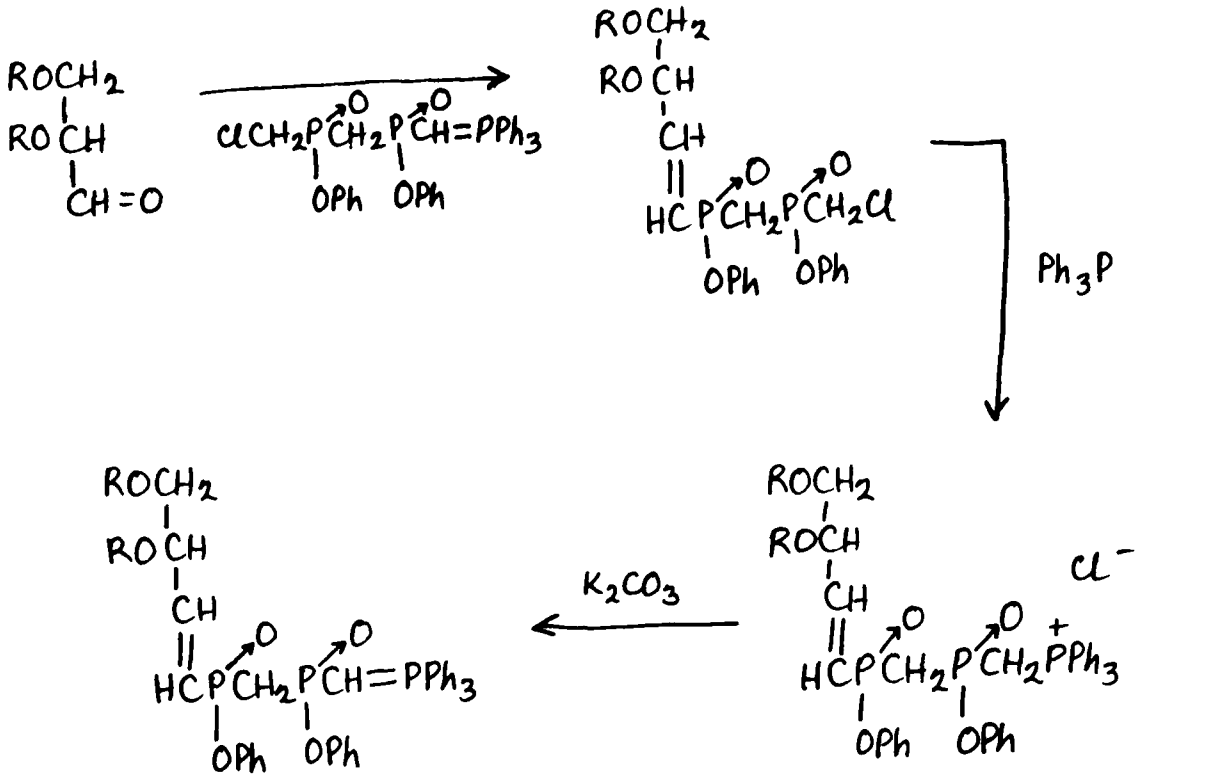
(a)



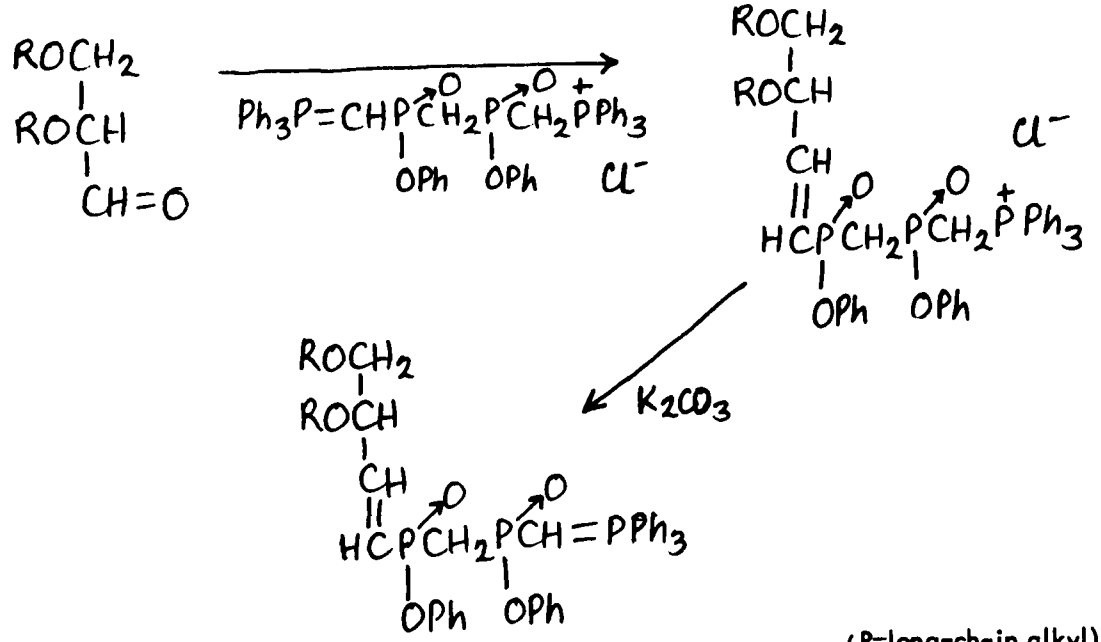
XXI

(b)

Fig 7



(a)



(R=long-chain alkyl)

(b)

Fig 8

ethanol or isopropyl alcohol to give the dihydrogen methylene-bis-phosphonite diester before subsequent reaction proved not nearly as satisfactory as the hydrolysis to the free acid.

Reaction of the acid with trioxane containing a little water and in the presence of a catalytic amount of trifluoroacetic acid gave a product which was adsorbed onto a column of Amberlite IR-45 resin in its trifluoroacetate form. The column was washed with water to remove formaldehyde and residual trioxane and the product was eluted with aqueous trifluoroacetic acid. The eluate on evaporation yielded a solid product which on dehydration with isopropyl alcohol gave a solid product in quantitative yield. Spectral data (n m r and infrared) and elemental analysis of this acid as its lead salt showed that this product was indeed the desired methylene-bis-(hydroxymethyl) diphosphinic acid. This highly encouraging result unfortunately occurred just prior to the conclusion of the contract period, so that additional attempts to form the corresponding bis-chloromethyl diphenyl ester, which was expected to be difficult (see below), had just been undertaken at the end of this period, without useful results from these preliminary experiments which could be reported so far.

4.4. Cytidine Diphosphate - Choline and - Ethanolamine Analogs.

The synthetic routes to compounds VIII and IX which were initially considered are outlined in schemes 9 and 10.

A considerable effort was put into this synthetic route during the earlier phases of this contract period. It had already been shown previously (14) that

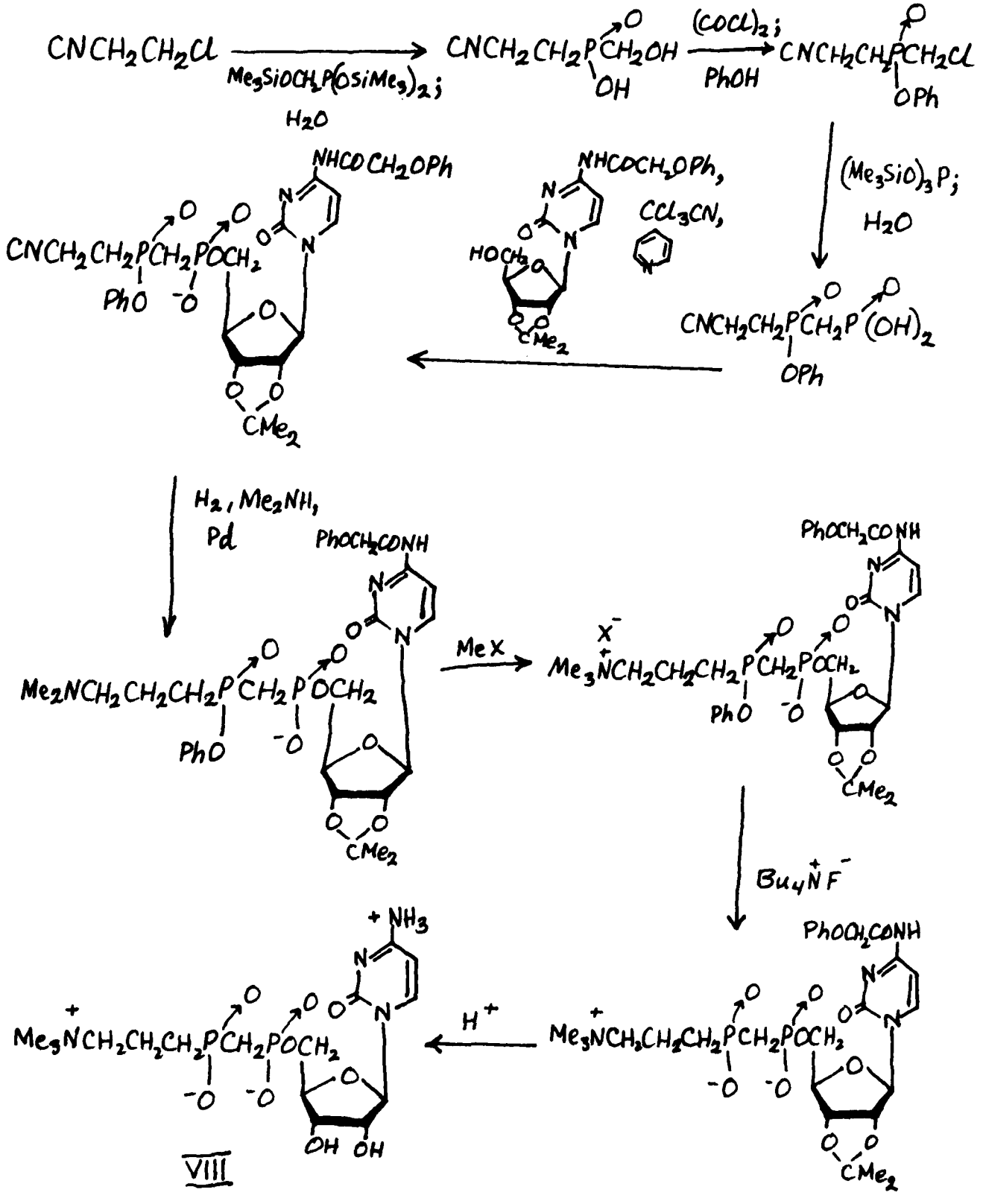

CNCH2CH2Cl
Me3SiOCH2P(OSiMe3)2;
H2O
CNCH2CH2P(O)(CH2OH)OH
(COCl)2;
PhOH
CNCH2CH2P(O)(CH2Cl)OPh
(Me3SiO)3P;
H2O
CNCH2CH2P(O)(CH2P(O)(OH)2)OPh
NHCOCH2OPh,
CCl3CN,
NHCOCH2OPh
CMe2
H2, Me2NH,
Pd
PhOCH2CONH
Me2NCH2CH2CH2P(O)(CH2P(O)(OPh)OCH2)
MeX
X-
Me3N+CH2CH2CH2P(O)(CH2P(O)(OPh)OCH2)
Bu4N+F-
+NH3
Me3N+CH2CH2CH2P(O)(CH2P(O)(O-)OCH2)
H+
VIII

Fig 9

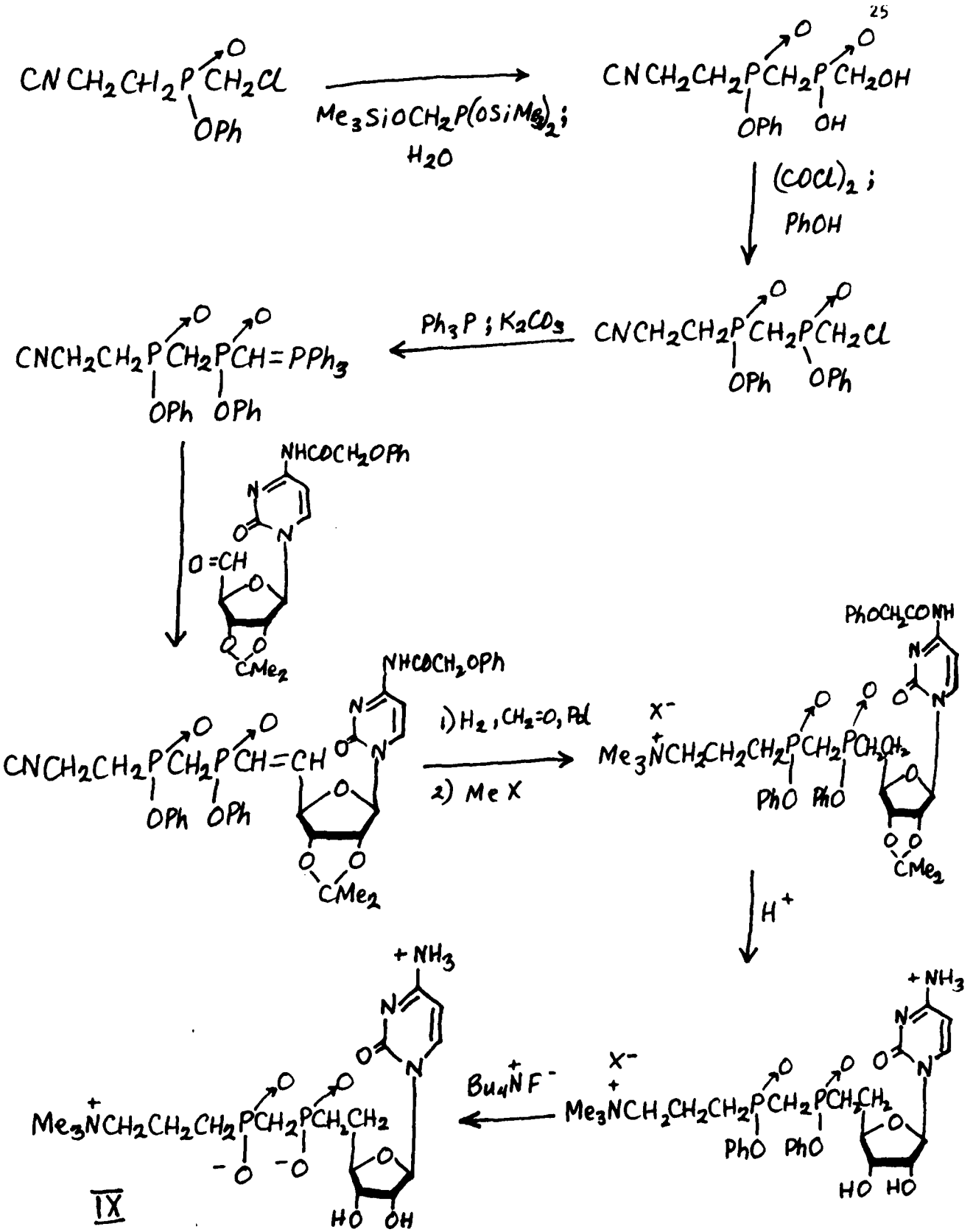

CNCH2CH2P(O)(OPh)CH2Cl
Me3SiOCH2P(OSiMe3)2; H2O
CNCH2CH2P(O)(OPh)CH2P(O)(OH)CH2OH
(COCl)2; PhOH
CNCH2CH2P(O)(OPh)CH2P(O)(OPh)CH2Cl
Ph3P; K2CO3
CNCH2CH2P(O)(OPh)CH2P(O)(OPh)CH=PPh3
NHCOCH2OPh
O=CH
CMe2
CNCH2CH2P(O)(OPh)CH2P(O)(OPh)CH=CH
i) H2, CH2=O, Pd
2) MeX
X-
Me3NCH2CH2CH2P(O)(OPh)CH2P(O)(OPh)CH2CH2
PhOCH2CONH
PhO PhO
H+
+NH3
Bu4N+F-
Me3N+CH2CH2CH2P(O)(O-)CH2P(O)(O-)CH2CH2
HO OH
IX

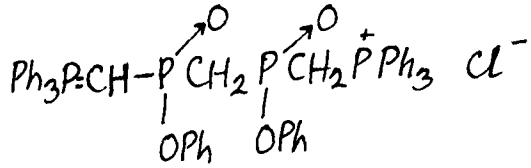
Fig 10

treatment of hypophosphorous acid with one mole of paraformaldehyde followed by silylation of the thoroughly dried oily mixture of products, would yield, among other substances, the requisite bis (trimethylsilyl) trimethylsilyloxymethylphosphonite, which would readily undergo Arbuzov reaction with 3-chloropropionitrile. Hydrolysis of the intermediate silyl ester-ether in water at room temperature gave the expected 2-cyanoethyl (hydroxymethyl)-phosphinic acid. The problem was next to chlorinate this acid at both the phosphinic acid and hydroxymethyl functions and produce a chloromethyl phenyl ester derivative. This proved unusually difficult, but eventually was accomplished by using oxalyl chloride followed by phenol in the absence of base. This work has already been reported in detail [b, e].

Attempts were made at this point to react the phenyl 2-cyanoethyl (chloromethyl) phosphinate with bis(trimethylsilyl) trimethylsilyloxymethylphosphonite, in a sequential application of the Arbuzov reaction with this rather unusual reagent. Preliminary indications for this reaction were that the expected bis-phosphinate could not be detected in appreciable amounts, although this was based on gas chromatographic analysis using a phosphorus - specific detector. It was anticipated that this compound, if not a low - melting solid, would certainly be an undistillable liquid. The experiment, as it turned out, was left in abeyance for the time being, because the analogous reaction with the diglyceride derivatives which served as a model synthetic system for the CDP-base analogs as well, was producing some difficulty at the time (see above) and more because of the publication of the synthetic route to methylene diphosphonous tetrachloride (see above). This promised a somewhat simpler synthetic

schema which is shown in figure 7.

This route, as indicated, depends upon the availability of the key Wittig reagent



for both the CDP-diglyceride and CDP-base analogs. Thus until this reagent became available, no work employing this route to the CDP-base analogs could be undertaken. For reasons discussed above, the liponucleotide analogs were the first ones to receive synthetic treatment by this route; and therefore no work specifically designed for the synthesis of the CDP-base analogs having no application to the CDP-diglyceride analogs was undertaken during the period of the contract beyond the previous progress report.

5. EXPERIMENTAL

Methylenediphosphonic Tetrachloride From Methylenediphosphonous Tetrachloride By Air Oxidation.

The preparation of the diphosphonic tetrachloride has not previously been reported in the literature by this method. A moderately vigorous stream of dry,

oil-free, filtered air was bubbled through methylenediphosphonous tetrachloride (0.4 ml) during 2 1/2 hours at room temperature. The reaction mixture gradually became solid and adhered to the walls of the flask. Anhydrous hexane was added to completely precipitate the product, which was filtered off under a nitrogen atmosphere under nitrogen pressure. In some experiments the product could be purified by crystallization from warm toluene, but in experiments in which only small quantities were used it was decided that this was not advisable due to the extreme sensitivity of the diphosphonic tetrachloride to hydrolysis. For use in subsequent reactions, the precipitate was dissolved in anhydrous, peroxide-free tetrahydrofuran.

rac-Glycerol-2-hexadecyl-3-octadecyl diether and condensation.

2-hexadecoxy-3-octadecoxyiodopropane (25g, 0.031 mol) was dissolved in 200 ml of hot dimethylformamide. Sodium benzoate (13.5g, 0.938 mol) was added, followed by 100 ml additional dimethylformamide. The mixture was heated to reflux and left under reflux overnight.

The semisolid reaction mixture was cooled to room temperature and mixed with ether; the inorganic salts were filtered off and washed with ether. Ether and then dimethylformamide were removed from the mixture by distillation and the residue extracted with ether and water to remove residual dimethylformamide. The ether phase was dried with magnesium sulfate, filtered, and evaporated to give 23g of crude product. Thin layer chromatography showed that little iodide remained and the product actually consisted mainly of the desired diether benzoate,

but additional by-products, both more and less polar, were present.

The crude product (23g) was dissolved with heating in 400 ml of propanol and 10 ml of a saturated solution of potassium hydroxide in 1:1 methanol-propanol was added. 500 ml more of propanol was added and the reaction mixture was heated on the steam bath for one hour.

The mixture was evaporated as well as possible in vacuo and extracted with ether and aqueous KH_2PO_4. The ether phase was dried with magnesium sulfate, filtered, and evaporated to give 22.9g of crude alcohol. Recrystallization from acetone yielded a pure product but with considerable loss of material; m.p. 55°; yield 8g (32%). The material was identical in chromatographic properties with that formed from reduction of the cleavage product of mannitol 1,2,5,6-tetraoctadecyl ether and periodic acid, using sodium borohydride.

The purified diether alcohol (240mg, 0.42mmol) in 2ml of anhydrous tetrahydrofuran containing 40 µl (0.6 mmol) of pyridine was added dropwise with stirring to the solution of methylenediphosphonic tetrachloride prepared above. A white precipitate appeared rapidly and the reaction mixture was left at room temperature for 48 hours and then heated to 40° for 1 1/2 hours. The white precipitate was filtered and to the filtered solution was added 0.5 ml of water and the mixture left for one hour at room temperature. The solution was evaporated and rendered anhydrous by re-evaporation several times with isopropyl alcohol. The residue was dissolved in a minimum amount of chloroform, in which it was very soluble, and precipitated by addition of an excess of acetonitrile.

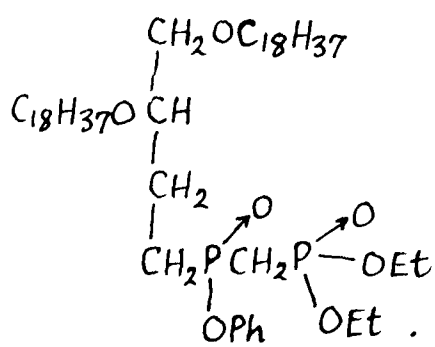
Thin layer chromatography of the filtered and dried product showed no starting materials remaining. The product weighed 0.26g after recrystallization from ethyl acetate.

This compound, however, failed to condense with the cytidine derivative as expected and thus was further investigated.

Phosphorus analysis showed 3.97% versus an expected 8.20%. This indicated that the material was primarily the bis-diether glycerol methylene diphosphinic acid, which would not be expected to condense with the cytidine derivative in pyridine solution. Molecular rate determination gave an approximate value of 1022; this is much higher than the expected tribasic acid (755.06).

In another experiment it appeared that a certain quantity of the expected liponucleotide analog was in fact formed, although still not the main product. Therefore, it appeared that the reaction would proceed in a more satisfactory way with some protection of the non-reacting acid groups.

Phenyl diethoxyphosphonomethyl (3',4'-dioctadecoxybutyl) phosphinate,



The phenyl chloromethyl phosphinate as a crude Arbuzov reaction product (0.4g) and 8 ml of triethyl phosphite containing a trace of hydroquinone were heated in an air-cooled reflux apparatus under a slow stream of nitrogen for 48 hours at 150-152°. Almost all the chlorine had disappeared from the reaction residue. Excess of triethyl phosphite was distilled off at 50-55° at water pump pressure, and the residue after cooling below room temperature was precipitated with acetonitrile. Thin layer chromatography showed a trace of starting material and the desired product, plus some more polar and less polar impurities. Phenyl absorptions were found clearly in the n m r.

The crude unsaturated diethyl ester was hydrogenated over a borohydride-reduced unsupported palladium catalysts. Hydrogenation was allowed to proceed at room temperature under 50 lbs. of pressure for 16 hours.

The catalyst was carefully filtered and washed with tetrahydrofuran and evaporated. The cooled residue was precipitated with acetonitrile. The filtered product, weighing 300 mg, was carefully reprecipitated from acetonitrile to give a product of Rf 0.15 in 9:1 chloroform - ethyl acetate. The n m r spectrum showed the expected phenyl absorption intact.

Analysis: Calculated: %C 69.19; %H 11.16; %P 7.00. Found: %C 68.82; %H 11.11; % P 7.07.

<u>De-esterification of phenyl diethoxyphosphonomethyl (3',4'-dioctadecoxybutyl) phosphinate.</u>

To 0.2g of the phenyl diethyl triester was added 10 ml of trimethylsilyl bromide,

in which the triester was readily soluble. The clear yellowish solution was stirred for two hours at room temperature and the excess of reagent was evaporated in vacuo. The residue, which was very soluble in tetrahydrofuran, was hydrolyzed with 5:1 tetrahydrofuran-water for one hour at room temperature.

After evaporation of the solution the residue was dehydrated by re-evaporation several times with isopropyl alcohol. The cooled residue was dissolved in a minimum amount of chloroform and precipitated with acetonitrile to give approximately 100 mg of product which failed to show any phenyl absorptions in the n m r. TLC and IR are characteristic of phosphorus acids containing a number of P-OH groups. The latter gave the typical broad shallow absorption between 2900 - 2700 cm^{-1} and the former showed the characteristic streaking with an Rf similar to that of dialkoxypropylphosphonic acids.

Condensation of this product with a protected cytidine derivative had just begun by the end of the contract period.

Typical condensation of trimethylsilyl phosphite-derived acid with protected cytidine.

The starting acid (1.5g) was obtained by Arbuzov reaction of phenyl 3,4-dioctadecoxybutyl (chloromethyl) phosphinate with trimethylsilyl phosphite, followed by aqueous hydrolysis, and finally hydrogenation with 10% palladium on charcoal in tetrahydrofuran as before. The crude acid was adsorbed on a column containing 160g of SilicAR CC-7 and eluded with chloroform and chloroform-methanol mixtures containing between 10% and 25% methanol. Eluates of the column were monitored

by TLC and the fractions containing similar products were combined and evaporated. Several fractions of acids were obtained in this way and each were separately condensed with the protected cytidine.

The general condensation procedure was as follows: each acid was dissolved by warming in anhydrous pyridine and to the clear solution was added a two fold excess of N-phenoxyacetyl-2',3'-isopropylidene cytidine dissolved in pyridine. To the clear solution was then added an excess (ca. 3 ml) of trichloroacetonitrile. The reaction mixture was heated to 70° and kept at this temperature for 48 hours, during which it became brown but remained clear.

After evaporation of excess pyridine and trichloroacetonitrile the residue was evaporated successively with methanol, isopropyl alcohol, and chloroform, and finally dissolved in a minimum amount of chloroform. The clear solution was precipitated with acetonitrile. The precipitates were freed of the cytidine derivative by repeated washing with 10:1 acetonitrile-methanol. From each condensation at least two products containing both cytidine (uv - absorbing) and phosphorus were found together with number of smaller products, varying with the history of the starting phosphonic acid.

The mixtures were subjected to hydrolysis with aqueous formic and especially trifluoroacetic acid under various conditions of temperature and time. In all cases at least two main uv-absorbing and phosphorus-containing products were found, which did not always appear identical on TLC. Typically two main products of Rf 0.75 and 0.53 were found; the model compound cytidine-5'-(2-hexadecoxy-3-octadecoxypropyl) phosphonate has an Rf of 0.7. The final products obtained

were separated by thick layer chromatography or by column chromatography. Whether or not elution of the products was followed by dialysis, compounds containing the expected elemental analysis could not be obtained from this series of reactions. In some cases the phosphorus analysis of what appeared to be a fairly pure product was as little as 0.8% (calculated, 4.6%).

An experiment was also performed in which a starting acid which showed no phenyl groups in the n m r was first reacted with a copper(II)-phenanthroline complex in an effort to chelate the two ionized phosphorus acid groups into a six-membered ring structure, and thus make the unionized phosphorus group in pyridine solution solely available for condensation with the cytidine derivative and thus hopefully simplify the reaction mixture. Unfortunately, however, no condensation at all was observed in this reaction.

Methylenediphosphonous acid.

Concentrated hydrochloric acid (100 ml) was cooled in an ice bath to 0°C. From a pressure equalizing dropping funnel was added 21.8g (0.10 mol) of methylenebis-phosphonous dichloride[f, g]. The rate of addition was controlled so that the reaction mixture remained at 0°C and that only a single phase was present in the reaction mixture with vigorous magnetic stirring.

Upon completion of the addition (but not until the reaction mixture was homogeneous) the solution was allowed to warm to room temperature. A few mg of yellow material were removed by filtration through glass wool. The HCl and

water were removed by evaporation at reduced pressure (bath temperature 40-45°C). N_2 was bled into evaporator. Degassed (boiled) water was added to the colorless syrup and the solution was again concentrated. Again, N_2 was bled into evaporator. Anhydrous 2-propanol was added to remove water by repeated azeotopic distillation at reduced pressure (bath 40°-45°). Yield of viscous, colorless syrup 14.5g (100%).

Since a little H_2O is needed for the next reaction, it is not necessary to completely dry the bulk of the sample.

Bis(hydroxymethyl) methylenediphosphinic acid.

To the free acid obrained from the previous reaction (14.4g) was added 30.g (5-fold excess) of formaldehyde in the form of trioxane. (Trioxane had been recrystallized from 2-propanol, filtered, and all solvent carefully removed under vacuum and a stream of N_2 gas.) A minimum amount of water (boiled and cooled under N_2 previous to addition) was added to give a homogeneous solution at 45°C. Two drops of trifluoroacetic acid were added, the solution was mixed to homogeneity and then left (sealed) in an oil bath for 24 hours at 45°C.

The viscous mixture was diluted with 100 ml of H_2O to give a more fluid solution. This solution was passed through a column of IR 45 ion exchange resin (previously washed with 10% trifluoroacetic acid and water) with an appropriate bed volume to adsorb all of the methylene-bis(hydroxymethyl)phosphinic acid. The column was then eluted with degassed H_2O to remove all of the formaldehyde and trioxane from the column. When no more formaldehyde could be detected

in the eluate, the column was eluted with 10% trifluoroacetic acid in water to remove the desired phosphinic acid. The column was eluted until no more phosphorus was detectable (Dittmer-Lester Spray) in the eluate. The eluate containing the phosphinic acid was then evaporated under reduced pressure to remove the water and the trifluoroacetic acid. Subsequent repeated azeotropic distillation with anhydrous 2-propanol gave an amorphous beige solid. Yield 20.0g (100%).

The product was conveniently analyzed as its insoluble lead salt, prepared by dissolving the acid in an acetate buffer, pH 5.5, and adding a solution of lead nitrate.

Calculated: %C, 8.80; %H, 1.97; %P, 15.17; %Pb, 50.63. Found: %C, 8.80; %H, 2.17; %P, 14.89; %Pb, 50.48.

COMPOUNDS SUBMITTED

(All Found Inactive in Standard Antimalarial Test)

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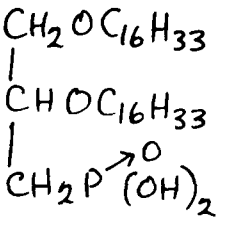

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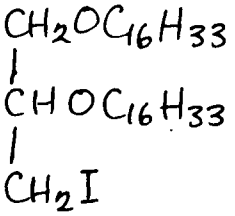

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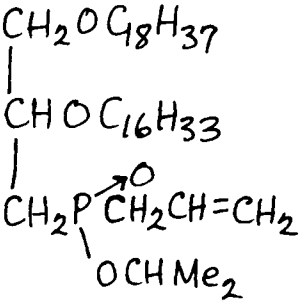

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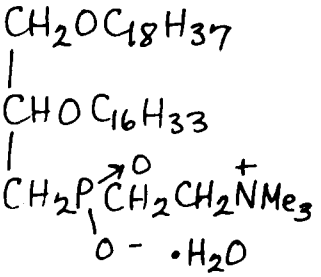

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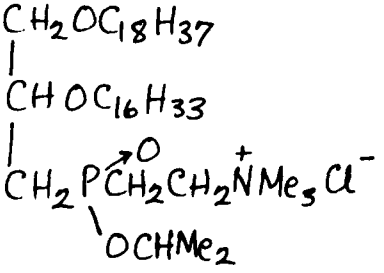

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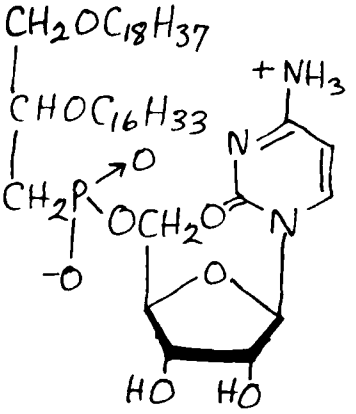

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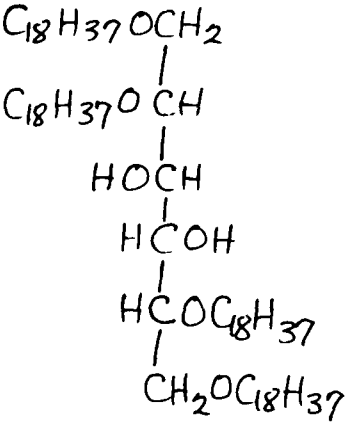

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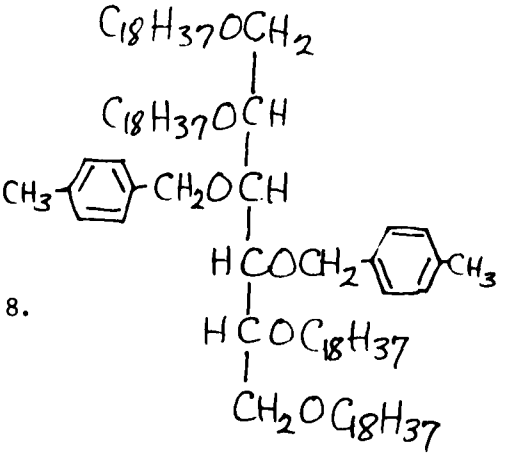

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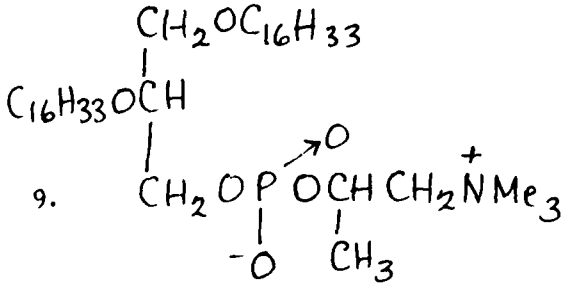

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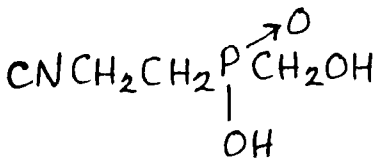

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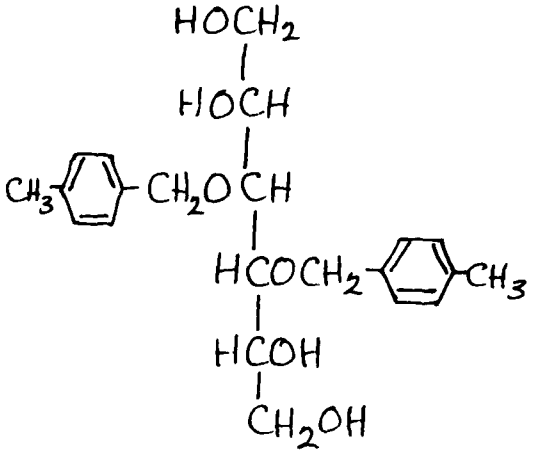

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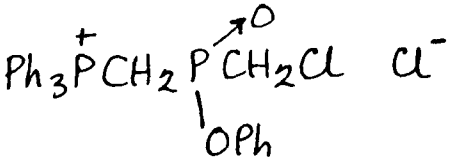

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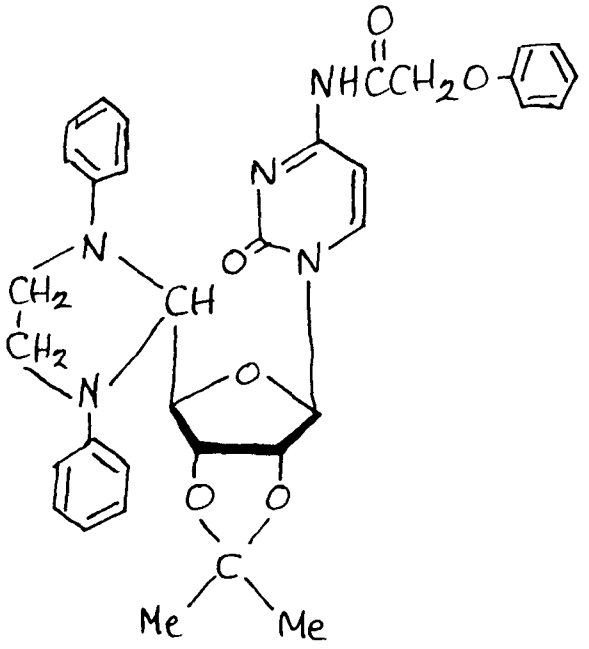

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REFERENCES TO PRIOR PROGRESS REPORTS

a - First Progress Report

b - Annual (Fourth) Progress Report, 1977

c - Second Progress Report

d - Third Progress Report

e - Fifth Progress Report

f - Sixth Progress Report

g - Seventh Progress Report

FIRST QUARTERLY REPORT: DAMD-17-76-C-6073

Contractor: Long Island Jewish-Hillside Medical Center

Principal Investigator: Dr. Arthur F. Rosenthal
Department of Laboratories
Long Island Jewish-Hillside Medical Center
New Hyde Park, New York 11040
212-470-2516

Period: July 1 — September 22, 1976

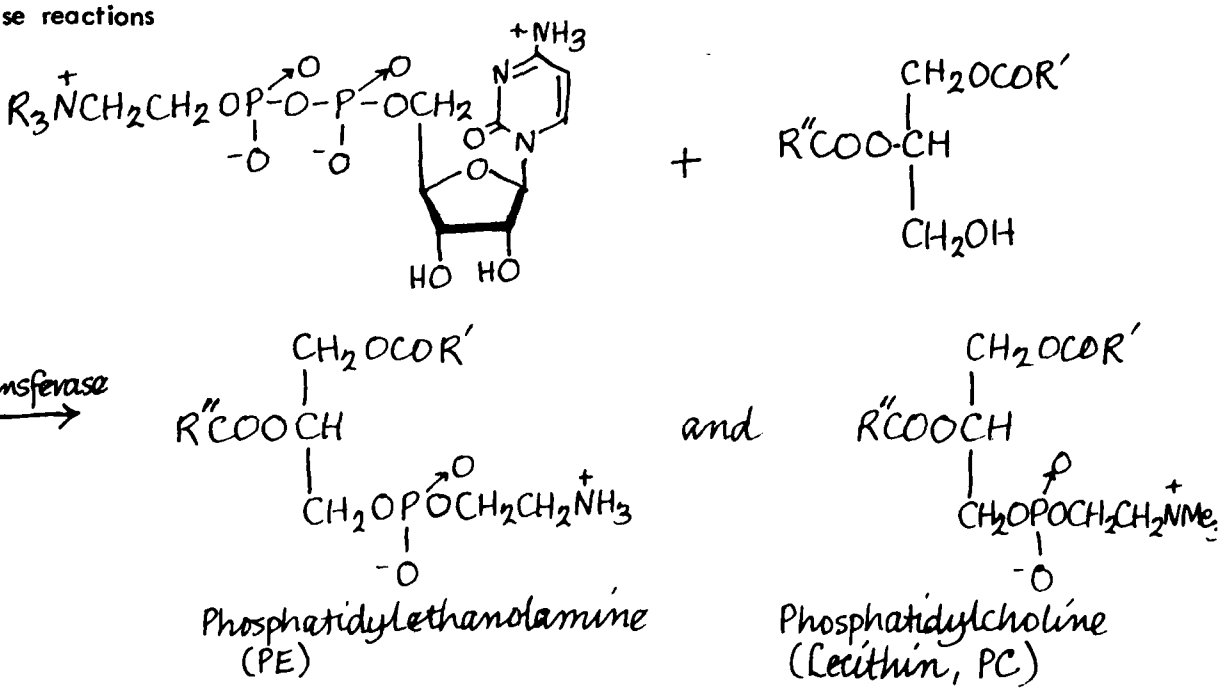
COMPOUNDS SUBMITTED:

(a) 2,3-Dihexadecoxypropylphosphonic acid ✓

(b) 2,3-Dihexadecoxyiodopropane ✓

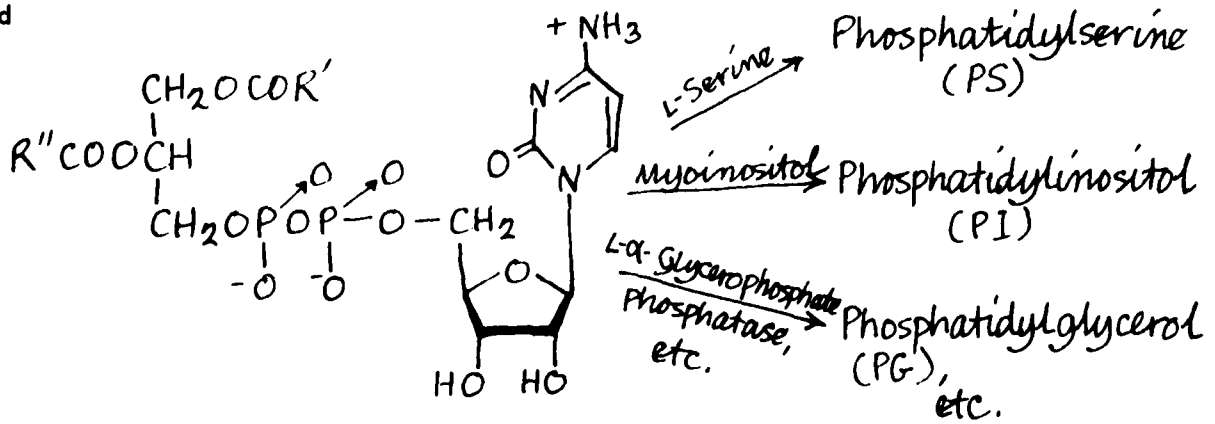
The present work has been designed to provide synthetic inhibitors of phospholipid biosynthesis which may prevent the growth and reproduction of Plasmodia and thus act as useful antimalarial agents.

It has been known for some time that Plasmodia, particularly in the well-studied intraerythrocytic stages, have a very active lipid metabolism compared to their host erythrocytes [1-3]. In recent years this has been shown to be particularly true of their phospholipid biogenesis, of which many details have been clarified. The transferase reactions



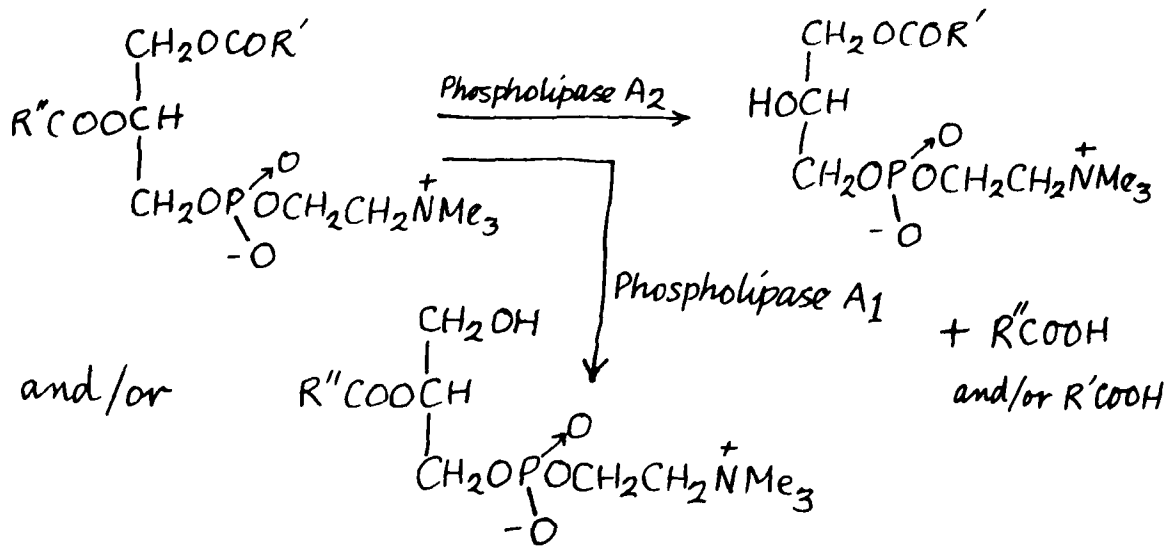
R = H or Me R′ and R″ = Long-chain alkyl

and



are apparently both highly active, since ethanolamine and choline are readily incorporated into plasmodial membrane PE and PC [2], and lipids produced by the latter pathway, such as PI and PG, are readily formed from labelled phosphate [3]. Phospholipids formed by both pathways are the major lipid components of plasmodial membranes [4, 5].

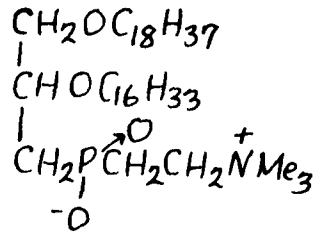
A possibly vulnerable but in any event highly significant point of plasmodial lipid metabolism is the inability of the organisms to synthesize fatty acids de novo; thus the source of fatty acids for this active phospholipid metabolism is the host erythrocyte and plasma lipids [2,3,6]. There exists no clearer example of chemical parasitism by these organisms. The erythrocyte membrane phospholipids are hydrolyzed rapidly by a phospholipase A:



This reaction and similar reactions with plasma phospholipid, as well as plasma free fatty acids, probably supplies the major portion of the fatty acid requirements for plasmodial growth and development.

During this project quarter the major effort has been directed toward the resynthesis in quantity of a lecithin analog which has previously been shown to be a good inhibitor of snake venom phospholipase A [7,8]. The rationale for this substance as a target compound is first of all an attempt to inhibit plasmodial phospholipase A and thus to deprive the parasites of their fatty acids for membrane phospholipid formation. Assuming a similar inhibitory activity toward plasmodial phospholipase A,

the nature of this target compound also provides a structure which should be biologically highly persistent because it possesses no points of hydrolytic lability:



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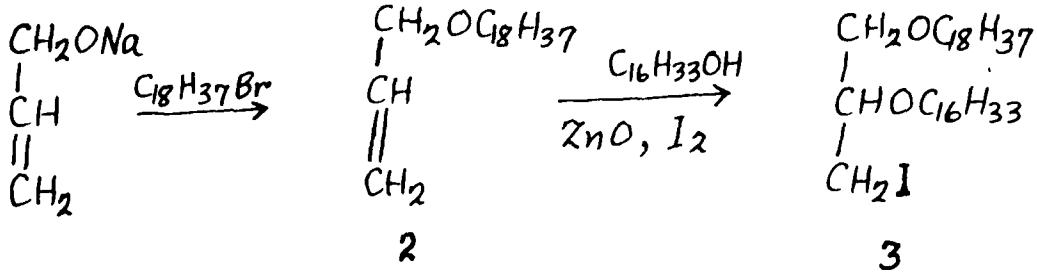
The ether moities which substitute for the ester groups in the natural phospholipid are probably much more slowly degradable than the latter. The C-P bonds are chemically of the same order of stability as C-C bonds and should be even more difficultly metabolized. In the absence of specific mechanisms to bring about a rapid excretion of this substance, it can be anticipated to possess a long duration of action should it show a desirable antimalarial activity. This substance has also been found (in unpublished preliminary experiments) to be very nontoxic to mice. The synthetic route is given below:



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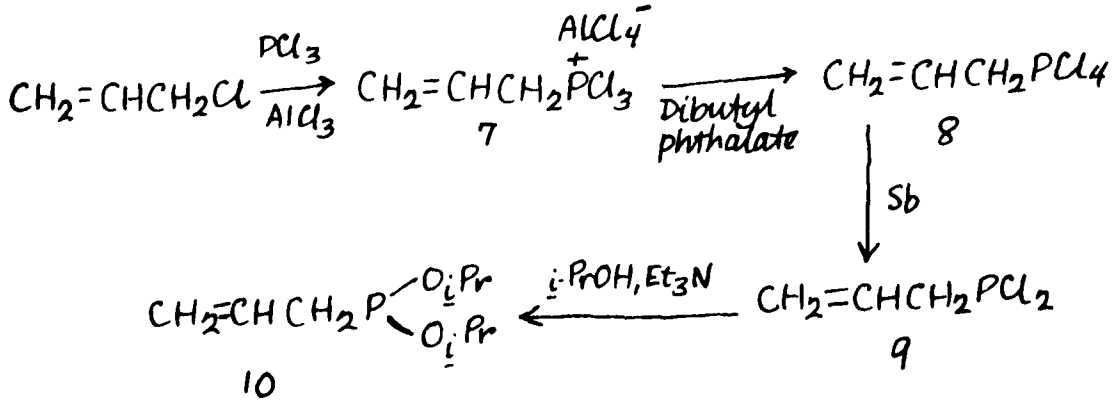
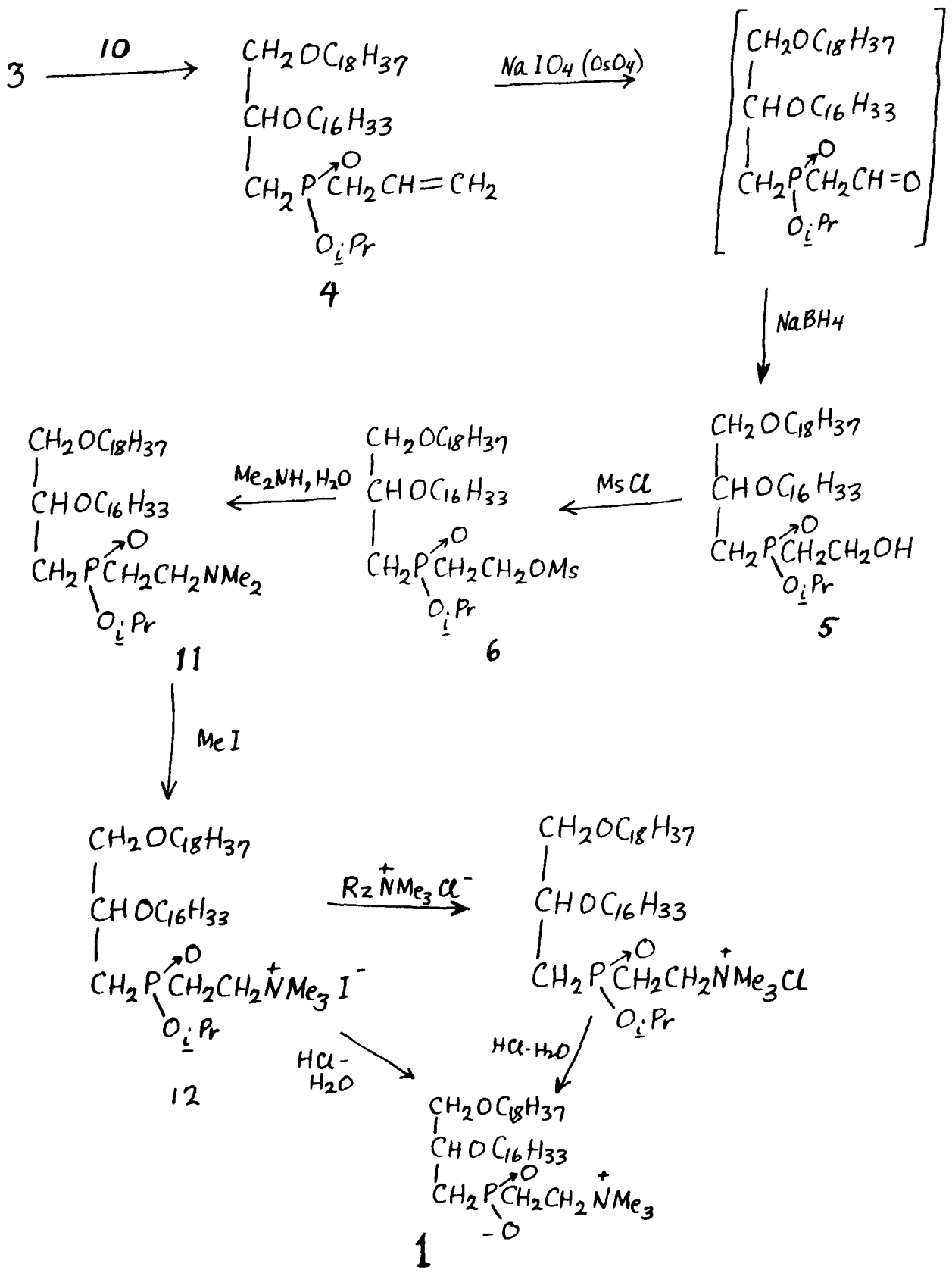


7 8 9 10


3
10
NaIO4 (OsO4)
NaBH4
MsCl
Me2NH, H2O
MeI
RzNMe3Cl-
HCl-H2O
HCl-H2O
4
5
6
11
12
1

The synthetic steps are discussed in detail below.

The preparation of allyl octadecyl ether from octadecyl bromide and sodium allyloxide in the presence of an excess of allyl alcohol presents no difficulties. The product, however, is contaminated with low molecular weight ethers and acetals and must be distilled in order to obtain a product of sufficient purity to be used in the next step. The formation of the iododiether (reaction 2 → 3) from allyl octadecyl ether is an interesting variant of the hypohalite addition reaction. A review of earlier literature indicates that hypohalite additions have hardly if ever been employed using long chain alcohols to provide haloethers. Furthermore, the reaction has almost always been carried out using mercuric oxide or silver oxide. In our laboratory a comparative study of this reaction and its optimal conditions was carried out sometime ago.

The conclusions reached are: (a) While mercuric oxide and cadmium oxide are very effective in the reaction, they are in no way superior to zinc oxide, which is of course far cheaper and less toxic; (b) the reaction is very sluggish at room temperature or below, but goes very well if moderate heat is applied; at about 55° overnight, yields approaching 80% can routinely be achieved; (c) iodine is superior to bromine in the reaction because it has much less tendency to halogenate other than the desired positions by radical mechanisms; (d) little if any of the 2-iodo-1,3-diether positional isomer can be detected, so that the reaction may be considered essentially completely positionally selective.

The reaction is also interesting in that it is one of the few synthetic reactions which one might run across in which 4 distinct reactants are employed, and which are made to react simply by mixing together, warming, and stirring in an inert solvent. Thus, a mixture of allyl octadecyl ether, cetyl alcohol, zinc oxide, and iodine are stirred together at 55° in 1,2-dimethoxyethane overnight. At the completion of the reaction the solvent is removed in vacuo; this is attended with some foaming because of the presence of unreacted zinc oxide, although a considerable amount of the oxide dissolves during the course of the reaction (the zinc iodide formed is very soluble in dimethoxyethane). The purpose of this evaporation is to allow subsequent washing of the product in ether solution with sodium thiosulfate to remove excess iodine; the presence of the water soluble dimethoxyethane would of course interfere with this

process. When the washing has been completed, as seen by the disappearance of the iodine color, the ether layer (from which of course most of the zinc oxide has been transferred to the aqueous layer by virtue of its high density) is now dried over magnesium sulfate and evaporated.

The reaction mixture at this point contains the product and excess cetyl alcohol. Advantage is taken of the insolubility of the product but the high solubility of cetyl alcohol in methanol at around 22°. The byproduct is dissolved in a relatively small volume of ether and a large excess of methanol is added; the almost pure product precipitates at once. Control of the temperature is very important, because the product has a melting point of about 28°, so that too high a temperature would precipitate an oil. The solubility of cetyl alcohol in methanol drops tremendously with only a few degree fall in temperature, so that too low a temperature fails to eliminate the cetyl alcohol from the product. There is probably no more than a 4 or 5 degree spread (probably between 19 and 23°) over which the product can be purified in this way. We have, however, never experienced any particular difficulty within this temperature range in obtaining a pure white granular product by this procedure at once. Recrystalization from ethyl acetate-acetone gives completely pure material. The compound is very soluble in very nonpolar solvents and very insoluble in moderately polar or polar solvents. Unlike homologs containing short chain ethers, this compound does not appear to be appreciably light sensitive and can be kept without difficulty for many years in a freezer.

The next step (3 → 4) is the most difficult in the entire synthetic sequence. It is not primarily that the Arbuzov reaction itself gives any great problem, although the known relative lack of reactivity of β-haloethers is perhaps somewhat in evidence. The primary problem is to obtain the requisite phosphonite reagent 10, which is synthesized with some difficulty and is very highly reactive so that it can not be kept for any significant period of time. The procedure follows that of Razumov, et al [9], but a considerably expanded number of experimental details are necessary in order to obtain the phosphonite 10 in reasonable yields.

One characteristic of the synthetic subsequence leading to this reagent (7 → 10) is that each succeeding intermediate is more labile than the one before; thus, the entire sequence of reactions must be accomplished together without delay. There are

two saving graces in this series of reactions; one is that the entire sequence can be completed within one week, although only by considerable concentration and effort; and the other is that the starting materials are cheap, thus overcoming the effect of the only moderate yields obtainable. The final product, if the reaction sequence has been properly carried out, can be obtained in very pure form. In this synthetic scheme allyl chloride is first reacted with a mixture of phosphorus trichloride and aluminum chloride to form the chloroaluminate complex of the allyl substituted phosphorus pentachloride 7. To the crude reaction mixture is added dibutyl phthalate, which has a greater affinity for aluminum chloride than the alkenyl phosphorus tetrachloride or, for that matter than the pi-complexed double bond of the allyl group. The liberated tetrachloride 8, like its parent phosphorus pentachloride, has 2 chlorines which are much more reactive than the rest. Antimony powder reacts to reduce these two specially reactive chlorines, liberating allylphosphonous dichloride 9. This is the first stage in the reaction sequence at which a product can be separated and purified. The dichloride must be distilled away from the much larger quantities of aluminum chloride complexes, excess dibutyl phthalate, antimony chloride, most troublesome, the large excess of phosphorus trichloride which remains from the first step. The purification of the product must be accomplished in two stages; the volatile materials (the product and excess phosphorus trichloride) are distilled from the rest of the reaction product; in a separate distillation the desired phosphonous dichloride is then separated from phosphorus trichloride. This must be done efficiently because excess phosphorus trichloride will react in the next step to form tri-isopropyl phosphite which will of course also react as an Arbuzov reagent. Both distillations must be conducted with the use of well regulated oil baths. The use of heating mantles is usually attended by pyrolyses leading to little or no product.

Allylphosphonous dichloride is a highly reactive substance relative to moisture and oxygen in the air. It must then be reacted with isopropanol in the presence of triethylamine to give diisopropyl allylphosphinate 10. Since this substance is even more reactive with atmospheric oxygen, every attempt must be made to minimize contact of the product with air, a procedure which is difficult because

the product must be filtered free of the byproduct triethylamine hydrochloride. The filtration must be as complete as possible, because in subsequent distillations triethylamine hydrochloride tends to volatilize and reform in the distilling receiver. This filtration can be fairly satisfactorily accomplished in a glove bag using rubber bulb suction or by nitrogen pressure transfer filtration from flask to flask through glass wool or a coarse fritted filter.

The product is obtained by removal of the solvent and distillation. For the final distillation of this product, as well as of its precursor 9, we use a Nester–Faust annular teflon spinning band column. Less efficient columns have always given poor final results. The Arbuzov reagent, once it is prepared, must then be reacted at once with the iodo-diether 3.

This is performed under a static or almost static nitrogen atmosphere in an apparatus which allows escape of the isopropyl iodide which is formed. Many ratios of Arbuzov phosphonite and iodo-diether have been tried; if one is willing to separate unreacted starting material, it seems to make little difference to the final result as long as the phosphonite is present in some excess. Since the phosphonite is by far the harder starting material to make, as much iodo-diether as is feasible is used in the reaction. The byproducts (besides isopropyl iodide) are largely the unreacted starting iodide and some low polarity apparent dehydrohalogenation products. The reaction indeed must be run at the rather unusually low temperature of 120 to 125°; if performed at the more common Arbuzov reaction temperatures (150-180°) these low polarity halogen-free products become the major reaction product and relatively little of the desired phosphinate 4 is formed.

Under our best conditions almost the only products are 4 and the starting iodide 3. The necessity for preparing the target compound 1 on a much larger scale than has previously been accomplished has led to a certain amount of investigation during this project period of alternative means of separation and purification of the product besides the open column chromatography which was previously used. Scaling up of the prior procedure would have necessitated the use of very large volumes of solvents, which it was desirable to avoid due both to cost and safety considerations. Furthermore, on such a large scale these procedures would have been slow as well as cumbersome.

Investigation on a small scale showed that dry column chromatography was a very satisfactory means of avoiding most of the difficulties associated with the previous chromatographic separation.

A dry column of silica gel containing fluorescent indicator was packed into a nylon tube which had been heat-sealed at the bottom according to the directions of Loev and Goodman[10]. With the solvent system employed the separation was very broad and therefore it was possible to grossly overload the column by the criteria of these authors. Although the desired product is not ultraviolet absorbing using the wavelength employed, the iodo-diether is highly absorbing and can be seen toward the bottom of the chromatogram. The product was located by puncture analysis[11]. In a large run, after the column was sliced it was most efficient to make wet columns for the elution from the adsorbent contained in the product-bearing fractions. Using this procedure a very large saving in both solvent and time was achieved.

The allylphosphinate 4 obtained from this reaction always consists of two essentially equal fractions which can be separated by TLC. These are apparently the diastereomeric forms of the product which are to be expected (optical isomerism about P as well as C 2).

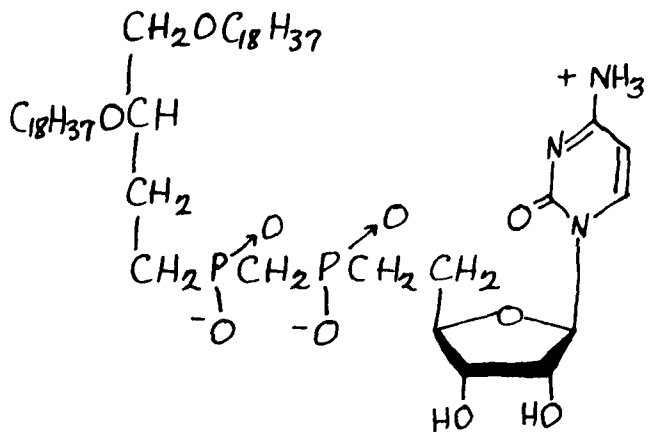
The remainder of the synthesis is fairly straightforward. Oxidation of the allyl double bond by osmium tetroxide-catalyzed sodium periodate gave the aldomethylene compound, which was not isolated but whose yield could be estimated from the ratio of the carbonyl to C-H bands in the infrared. In the large scale reaction it was necessary to substitute tetrahydrofuran, which is a much better lipid solvent, for much of the ethanol which was previously used on a smaller scale. There was evidently no disadvantage to using the solvent mixture. Reaction with sodium borohydride then yielded the alcohol 5. Treatment with methanesulfonyl chloride in pyridine gave the desired mesylate ester without difficulty. The introduction of the trimethylammonium function must be done in 2 steps, since the reaction of trimethalamine with β-halo phosphonates (and presumably phosphinates) gives only very rapid dehydrohalogenation. In aqueous solvents addition of dimethylamine to this presumably initially formed double bond (Michael addition) very quickly gives the dimethylamino compound 11. Quaternization with methyl

iodide yields the iodide salt 12. Incidentally, the insolubility of this quaternary iodide in cold ether provides a very convenient method of purifying the product away from nonpolar lipid contaminants.

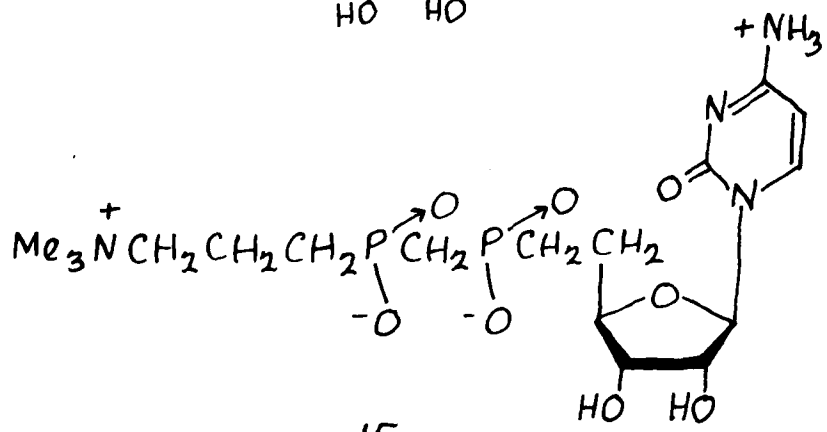
At the present writing (9.22.76) we are in the process of converting the iodide salt into the corresponding chloride for purposes of testing this ester or the target compound into a form which can be used for antimolarial testing. Simultaneously, we are taking the crude chloride and hydrolyzing it to give the target compound 1. Hydrolysis of the chloride is performed in acetic acid solution containing a relatively small amount of 6 N hydrochloric acid. Hydrolysis is carried out for at least 24 hours at 70°; these reasonably mild conditions have been found to be satisfactory, and are used to minimize degradation of the target compound. It can best be anticipated that within a short time both the isopropyl ester chloride and the target compound itself will be available in sufficient quantity for antimolarial testing.

The target compound 1 is a white crystalline solid which, as follows from its structure, is extremely resistant to acid hydrolytic treatment. However, when heated with base, it undergoes extensive degradative alterations, probably beginning with a deamination. The activated double bond thus exposed can be expected to undergo a variety of further reactions under these conditions. At ordinary temperatures and except in the presence of strong base the substance is very stable. One of its most remarkable characteristics is the great ease with which it is dispersed in water. Sonication for less than a minute at room temperature will produce an almost water clear dispersion. Comparison with natural lecithin of approximately the same chain-length, or with the related phosphonate lecithin analog, shows that the phosphinate is far more readily dispersible in water. This would not be anticipated <u>a priori</u>, because of the absence of slightly polar groups in the phosphinate analog which are present in lecithin. The substance is also somewhat less soluble in very nonpolar solvents than is a similar chain-length lecithin. The best solvent for compound 1 is a mixture of chloroform and methanol. The reason for this dispersive hydrophilicity and relative polarity compared to natural lecithins is at present unknown.

Although the synthesis of the phosphinate lecithin analog 1 was the major target during this furst quarter, some work was directed toward the cytidine diphosphate diglyceride analog 14 and the cytidine diphosphate choline analog 15:



14



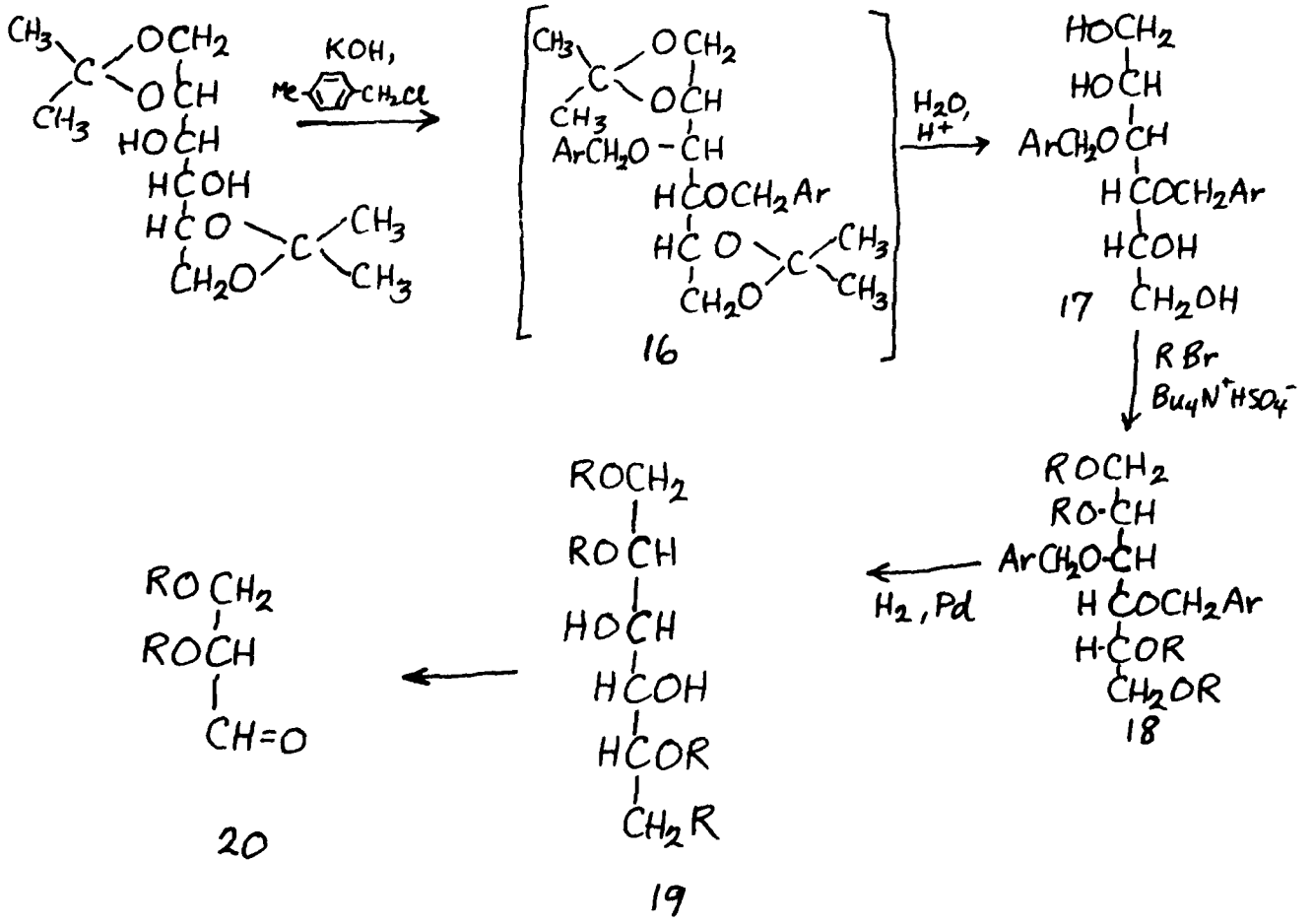
15

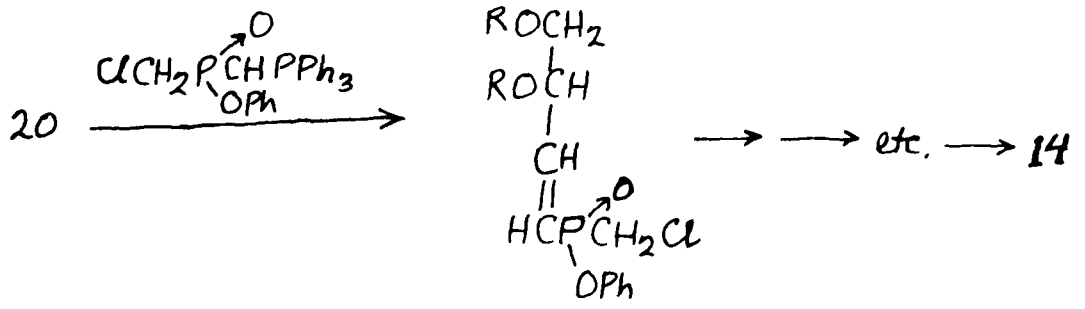
These syntheses are complex and for a complete outline the reader is referred th the original contract proposal. Only the portions on which work was done during this past quarterly period will be discussed here in any detail. The synthetic routes to these two compounds are fairly similar, and a large part of the synthetic scheme has actually been realized. For technical reasons the synthesis of 14 is to be undertaken first, which should allow many points of the synthetic route common to both analogs to be worked out with greater facility than would be the case if the two

syntheses were carried out exactly simultaneously.

The present necessity for preparing these analogs on a moderately large scale has first of all necessitated during this first project quarter the preparation of larger amounts of initial intermediates than were previously available. Particularly this has meant the resynthesis of the intermediate D-mannitol 1,2,5,6-tetraoctadecyl ether 19, which was prepared in our laboratory sometime previously[15], by modified methods which would allow easier handling of the larger amounts of material presently necessary. This intermediate is a direct precursor of L-glyceraldehyde 2,3-dioctadecyl ether(20; corresponding to intermediate XVII in the contract application, page 14).

The synthetic scheme is shown below:





The synthesis of the intermediate 16 and its deacetonation product mannitol 3,4-di-p-methylbenzyl ether 17, present no particular difficulties, although there is some problem in purifying 16 because when somewhat impure it is difficult to crystallize. A reasonably intensive attempt was made to purify the approximately 85% pure crude product by zone melting, but this proved completely unsuccessful even after many passes. When carefully crystallized first from hexane—chloroform, followed by aqueous methanol, however, very pure product can be obtained. Apparently the first crystallization removes very nonpolar impurities like bis-p-methylbenzyl ether while the aqueous crystallization removes mannitol 3-p-methylbenzyl ether, essentially the only impurity more polar than the product. In any event crystallization of 17 is far easier than crystallization of mannitol 3,4-dibenzyl ether, which often can be made to solidify only after many weeks or months in the refrigerator, freezer, or at room temperature with or without an appropriate crystallizing solvent. Therefore, even though the later removal of the p-methylbenzyl groups by hydrogenation is more difficult than the removal of benzyl groups, the greater ease of purification of the p-methylbenzyl diether more than compensates for this disadvantage. One of the improvements made in the synthetic route during the present quarter, in fact, related to a more effective and in fact more economical hydrogenolysis of these p-methylbenzyl groups so that currently the previous disadvantage can be considered essentially overcome.

The most difficult synthetic step which was studied in this series during this quarter relates to the formation of the hexaether 18 (17 → 18). In the method originally employed a large excess of octadecyl bromide is mixed with 17 plus a large amount of powdered potassium hydroxide and some calcium hydride (as a

dehydrating agent) in dioxane or 1,2-dimethoxyethane, and the mixture is very vigorously stirred mechanically just below the reflux temperature of the solvent for a number of hours. Excess potassium hydroxide and other calcium and potassium compounds are removed by filtering the mixture hot; filtration is often quite difficult. The product is isolated after removing the solvent in vacuo, taking it up in ether, and washing with water. Sometimes filtration is so difficult that it is necessary to remove the solvent first, then add ether and wash with water a number of times. The crude product obtainable at this point always is a complex mixture containing, besides the desired compound, a variety of partial ethers of mannitol as well as a large amount of dioctadecyl ether and excess octadecyl bromide. The method gives moderately low yields as well; even after extensive purification the product is very difficult to obtain free from closely related lower ethers. In the method developed during this project period an application of the phase transfer Williamson synthesis recently described by Freedman and Dubois[12] was investigated. Since these authors used the method to prepare only simple ethers, the preparation of the hexaether 18 containing 4 long chain groups seemed to offer a very rigorous test of this procedure.

The use of the alkyl chloride (in this case octadecyl chloride) as recommended by these authors gave very incomplete reaction even after 5 days at 80°. The substitution of octadecyl mesylate instead of a halide appeared to give satisfactory reaction, but the presence of excess mesylate greatly complicated the purification. The use of octadecyl bromide, however, gave very satisfactory reaction and no difficulties other than the formation of appreciable dioctadecyl ether, for which a good method for removal was developed. The use of a two-phase all-liquid system allows a much less cumbersome experimental procedure in which ordinary magnetic stirring is used. However, a somewhat unusual solvent mixture must be used for the organic phase in order to keep all components in solution (isopropyl ether-tetrahydropyran). The crude product obtained in this reaction is relatively simple and consists of a very nonpolar fraction (octadecyl bromide and possibly dehydrohalogenation product), dioctadecyl ether, the desired hexaether, and one more polar component, which may be a lower ether or an oxidation product. It was decided to purify 18 to homogeneity at this step, since it was felt that extensive purification of the aldehyde 13 could

thereby be avoided. Addition of absolute ethanol to the crude product at room temperature removed octadecyl bromide completely from the product. Solution of the resultant precipitate in warm hexane followed by cooling to about 8° precipitated the bulk of the dioctadecyl ether. A chromatographic step at this point appeared to be the only feasible method of separating the remaining components.

As in the lecithin analog described above, it was desired to avoid the use of very large amounts of solvent. After a good deal of trial and error experimentation, a very satisfactory dry column method of purification was developed. A hybrid alumina-silica column was found to give best results. The former adsorbent is necessary to separate the polar component from the rest, but is poor in resolving the nonpolar fractions of the mixture. Conversely, silica resolves the product from residual dioctadecyl ether. Unexpectedly, Woelm (ICN) dry column silica was found to be unsatisfactory in achieving this separation, but Baker silica gel achieved a very large separation of the two components, amounting to a virtually direct scale up of the TLC conditions. The product obtained by elution of the appropriate portion of the column (as determined by puncture analysis) after slicing was chromatographically homogeneous.

Hydrogenolysis in the previous procedure was found to be very slow, using a palladium on charcoal catalyst. In the present procedure a borohydride-reduced unsupported palladium catalyst was employed. A first portion added at the beginning of the reaction produced only very incomplete hydrogenolysis. However, a second portion of catalyst equal in amount to the first and prepared in the same way gave a rapid and complete hydrogenolysis of the p-methylbenzyl group. Apparently, the first portion of catalyst achieves the removal of trace poisons present in the reaction mixture. The product obtained after filtration of the catalyst, evaporation of the solvent, and precipitation with acetonitrile was essentially homogeneous and required no further purification. The palladium catalyst, having no major contaminants present, was simply dissolved in nitric acid and saved for future reduction and reuse. The nitric acid treatment has previously been found also to remove trace poisons from the recycled catalyst.

EXPERIMENTAL

Allylphosphonous dichloride, 9. Into a 5 l three-neck flask was placed a reflux condenser, a mechanical stirrer, and a double-neck adaptor into which were placed, respectively, a thermometer and a dropping funnel. At the top of the dropping funnel was placed a nitrogen inlet and a nitrogen outlet via a Bunsen valve was placed into a drying tube which fitted into the top of the condenser. Into the flask were placed anhydrous aluminum chloride (400.5 g) and phosphorus trichloride (524 ml). Into the dropping funnel was placed allyl chloride (122 ml) and the entire apparatus was swept carefully with nitrogen, avoiding appreciable evaporation of the volatile material. The allyl chloride was added dropwise just to maintain the temperature between 40 and 50 degrees while continuously stirring. The addition took approximately 4.5 hours. Toward the end of the addition the heat liberated was not sufficient to maintain the temperature and an oil bath was required. After the completion of the addition the temperature of the oil bath was raised to 90 $\pm 5^{\circ}$; the temperature within the reaction slowly approached that of the boiling point of phosphorus trichloride (76°). After maintaining this temperature for approximately 1 hour, it was seen that almost all the aluminum chloride had dissolved.

The mixture was cooled to room temperature and 525 ml of methylene chloride was added to dissolve the complex. Still maintaining the reaction mixture under nitrogen, 1.5 l of dibutyl phthalate was added dropwise with vigorous stirring. The first 500 ml was added slowly because the reaction is exothermic; gradually the rate of addition could be increased, however, and the last portion of dibutyl phthalate added quite rapidly.

The mixture was cooled to room temperature and powdered antimony (180 g) was carefully added while a slow stream of nitrogen was passed through the mixture. The reaction with antimony is somewhat exothermic, but when the initial heat had dissipated the mixture was stirred in an oil bath at $80\text{-}90^{\circ}$ for 4 hours, and then allowed to stand at room temperature overnight.

The following day the methylene chloride was distilled off at atmospheric pressure, collecting the fraction boiling below 45°. Approximately 350-400 ml of solvent was collected. The mixture of phosphorus trichloride and allylphosphonous dichloride was then distilled in vacuo using a water pump with an in-line drying tube to prevent entry of water vapor into the system. Using the oil bath, the temperature was gradually raised to 135-140°, and the distillate without fractionation was collected in a receiver which was almost completely submerged in an ice-methanol bath. The distillation operations up to this point occupied most of a working day. The mixture of product and excess phosphorus trichloride was kept protected from moisture at 4° overnight.

The mixture was redistilled through an efficient column at water pump pressure. The phosphorus trichloride was removed below room temperature and the product fraction boiling at 45 to 50° at 47 mm was collected. To remove any traces of phosphorus trichloride, the product was again redistilled and the fraction boiling at 40-41° at 25 mm was finally taken as the pure product. It weighed 96 g (44.5% yield based on 1.50 mol of allyl chloride).

Diisopropyl allylphosphonite, 10. Under a nitrogen atmosphere and with efficient mechanical stirring a solution of 96 g of allylphosphonous dichloride in 100 ml of anhydrous ethyl ether (distilled from calcium hydride) was added to a solution of 81 g of isopropyl alcohol and 136 g of triethylamine in 1200 ml of ether at -10 degrees. The dropwise addition was carried out during 1.5 hours, after which the mixture, with vigorous stirring, was heated under reflux for 2.5 hours. The mixture was allowed to cool and filtered under nitrogen in a glove bag from the large precipitate of triethylamine hydrochloride. The filtration is often difficult, and in some preparations an appreciable amount of salt remains in the filtrate. If this occurs it is best to distill the ether off at atmospheric pressure and (keeping air away from the product) to dissolve the residue in pentane, repeat the filtration, and again evaporate the solvent at atmospheric pressure. Pentane is a poor solvent for all the reaction components and cannot adequately be used as the reaction medium for this reason.

The product was then distilled, using a high efficiency spinning band column with an oil bath heated to no more than 120°, and water pump vacuum. The fraction boiling at 75 to 80° at 20 mm was taken as diisopropyl allylphosphonite. This product weighed 67.5 g (51%). Experience suggests that the yield in the reaction flask is actually con-

siderably higher, but an appreciable proportion of the product is oxidized during the inevitable contact with some air during filtration and other manipulations; a considerable higher boiling fraction remains in the pot, representing largely this oxidation product (diisopropyl allylphosphonate). Indeed, when the phosphonite is spread out in the thin layer in air this oxidation takes place with almost explosive rapidity, liberating a good deal of smoke and heat.

Diisopropyl allylphosphonite should be used at once without any attempt at prolonged storage.

DL-isopropyl 2-hexadecoxy-3-octadecoxypropyl(allyl)phosphinate, 4. 2-Hexadecoxy-3-octadecoxyiodopropane was prepared exactly as given in reference[13], but with a scale up to dekagram quantities (a homolog of this intermediate, 2,3-dihexadecoxyiodopropane has been submitted to WRAIR as a trial antimalarial).

2-Hexadecoxy-3-octadecoxyiodopropane (98 g, 0.145 mol) and 68 g (0.36 mol) of diisopropyl allylphosphonite were heated under a static nitrogen atmosphere, with provision for removal of isopropyl iodide, at 120-122° for 40 hours. At the end of this time, the clear solution was virtually free of iodine. After cooling, the mixture was dissolved in hexane and the solution washed with dilute hydrochloric acid and then water. The hexane phase was dried over magnesium sulfate, filtered and evaporated in vacuo. To the residue was added cold acetonitrile in large excess; the exact quantity is not important, because the product has virtually no solubility in the solvent, which is used to remove low molecular weight impurities (particularly phosphorus compounds). After drying in vacuo the crude product weighed 82 g. It consisted essentially of the diastereomeric racemate of desired product and iododiether starting material, plus possibly some additional highly nonpolar byproducts in small amounts.

Past experience suggested the desirability of purifying this intermediate before further steps were undertaken. The crude product (25 g) in a minimum quantity of methylene chloride was applied to the top of a dry column of Woelm (ICN) dry column silica (500 g) resting on glass wool in a nylon column which had been sealed at the bottom and punctured with a needle to provide air outflow [10]. The column was developed with about 400 ml of 2% ethanol in methylene chloride. Compounds contained on the column were detected by puncture analysis [11]. Every 2 cm, starting at the top, a small sample of silica was removed and analyzed by TLC after elution by

20% ethanol in methylene chloride (silica gel G; 6% ethanol in methylene chloride). The product was found in fractions 12-24 along the length of the column. The starting material as well as possible other nonpolar compounds was found in fractions 34-46. This fraction could be easily visualized under short wave length ultraviolet light as well, although the product fractions were essentially invisible. Although the ratio of product to adsorbent grossly overloads the column, the separation is easily sufficient to overcome the consequent poor resolution; i.e., the highly favorable separation in this system can be used to separate the desired compound easily even under conditions where each individual compound is somewhat spread out. The fractions containing the desired product were eluted, after slicing the column, with 20% ethanol in methylene chloride. The solvent was removed in vacuo and pure product was obtained by precipitation with acetonitrile, as with the crude product; yield 14.7 g (59%). An additional 34 g of crude product was purified in this way, giving 22 g of pure product. The two diastereomers were approximately equal in amount and had Rf's 0.58 and 0.64 on TLC.

DL-Isopropyl 2-hexadecoxy-3-octadecoxypropyl(2'-hydroxyethyl)phosphinate,5. The allyl compound (10 g, 0.0147 mol) was dissolved in tetrahydrofuran (300 ml) and added to a tetrahydrofuran-water solution (9:1) to which osmium tetroxide (0.356 g, 0.0014 mol) in carbon tetrachloride had been added. Sodium metaperiodate solution (10 g in 300 ml water) was added drop wise over a period of 1 hour. The color of the solution changed from brown to yellow. A white solid (sodium iodate) slowly precipitated. The reaction was allowed to stand overnight at room temperature, and then evaporated in vacuo (bath 34-37°) until almost all the ethanol had been removed. The mixture was extracted with chloroform; the combined chloroform extracts were dried over magnesium sulfate and filtered. The solvent was removed in vacuo; The grayish product (10 g) had a very strong carbonyl band at about 1710 cm^{-1}. This modified oxidation is based on the work of Baggaley, et al [14].

To the crude aldehyde (10 g) in 330 ml of ethanol was added sodium borohydride (4.3 g). The mixture was stirred at room temperature for 15 hours and then cooled to 5°. Hydrochloric acid (12 m) was added slowly with stirring to the reaction mixture until all the borohydride was destroyed (about 10 ml). After concentration of the reaction mixture almost to dryness (bath temperature 40°) the reaction mixture was

extracted with chloroform and water. The chloroform layer was washed twice with water, dried with magnesium sulfate, filtered and evaporated to dryness in vacuo (bath 40°). The crude alcohol (9.14 g) was obtained as a grayish colored solid.

DL-Isopropyl 2-hexadecoxy-3-octadecoxypropyl(2'-mesyloxyethyl)phosphinate, 6. The crude alcohol (9.14 g) in anhydrous redistilled pyridine (180 ml) was cooled to 0-5° and methanesulfonyl chloride (21 ml) was added dropwise during 10 minutes with vigorous magnetic stirring. The stirring was continued in the cold for 20 minutes and the reaction was completed by stirring at room temperature for an additional 30 minutes. To the reaction mixture, again cooled to 0-5°, was added ether (700 ml) and 350 ml of water dropwise. The mixture was transferred to a separatory funnel, the phases separated, and the aqueous phase washed twice with 300 ml portions of ether. The combined ether extract was washed with water, 1 M sulfuric acid, 3% aqueous sodium carbonate and finally again with water. The ether solution was dried over magnesium sulfate, filtered and evaporated in vacuo (bath 40°). Crude yield, 11.0 g. The spots at Rf's 0.78 and 0.86 in 8% ethanol in methylene chloride possibly represent diastereomeric forms of the product.

DL-Isopropyl 2-hexadecoxy-3-octadecoxypropyl(2'-dimethylaminoethyl)phosphinate, 11. To 11.0 g of the crude sulfonic ester in tetrahydrofuran (460 ml) was added 208 ml of 40% aqueous dimethylamine and 164 ml of water. The mixture was stirred at room temperature overnight and concentrated in vacuo (bath 40°). The residue was dissolved in ether and swirled carefully with a solution of 40% potassium carbonate in 0.1 M aqueous sodium hydroxide without vigorous shaking. The layers were allowed to separate completely and the aqueous layer was removed. This solution on evaporation yielded 9.6 g of crude dimethylamino product.

DL-Isopropyl 2-hexadecoxy-3-octadecoxypropyl (2'-trimethylammonium)ethyl phosphinate, 12. A quantity of the ethereal extract from the previous preparation containing 7.4 g of crude dimethylamino compound and 5 ml of methyl iodide were kept in the dark at room temperature for 4 days. The mixture was cooled to about 15° for a few hours and filtered, the precipitate was washed thoroughly with minimal quantities of cold ether and the product dried in vacuo. The yield of iodide was 6.6 g. An average yield of this product from intermediate 5 is as high as 47%. This material could be purified by crystallization from chloroform-acetone. The pure material has melting

point 166-167° with decomposition. The product moves as a single spot in chloroform methanol water, 65:25:4. Rf's have ranged from 0.31 to 0.5 with different preparations of silica over a period of several years.

Succeeding steps. The hydrolysis of the isopropyl ester to the target compound 1 has of course been previously accomplished [7] and is at present underway on a larger scale. The ion exchange of iodide 12 to chloride 13 is also under study for the preparation of a possibly interesting potential antimalarial intermediate. This ion exchange has so far been found to be not as straightforward as anticipated, and to require additional study of conditions for optimal yield. It is anticipated that both these compounds will shortly become available for submission to WRAIR.

D-Mannitol 1,2,5,6-tetraoctadecyl-3,4-di-p-methylbenzyl hexaether, 18. D-Mannitol 3,4-di-p-methylbenzyl ether was prepared by the method previously reported [15] and purified as indicated in the text above. The diether (12.15 g, 0.031 mol) was dissolved in a mixture of 100 ml of tetrahydropyran and 250 ml of diisopropyl ether, both peroxide-free. To the solution was added 1-bromooctadecane (125 g), followed by aqueous potassium hydroxide (500 ml solution containing 325 g KOH) and 2.5 g of tetrabutylammonium bisulfate. The mixture was magnetically stirred at 80° for 72 hours. Shorter reaction times result in appreciably larger amounts of incomplete etherification products. The crude mixture was diluted with water and ether and extracted. The organic phase was washed with concentrated aqueous sodium chloride, dried over magnesium sulfate and evaporated. To the residue was added a little ether just until it was completely dissolved, and the product precipitated with 10 volumes of 99% ethanol. It was filtered isothermally; the filtrate contained almost none of the desired product but almost all of the octadecyl bromide plus some lower ethers. This crude material weighed 58 g, but most of the weight consisted of dioctadecyl ether, the major byproduct.

Ten g of the crude product was dissolved in hexane (100 ml) with warming, and the solution allowed to cool to 22°. A precipitate appeared which was filtered off isothermally and found to be essentially pure dioctadecyl ether (1.9 g). Approximately 1 g more of dioctadecyl ether can be obtained by cooling the filtrate to about 10°, but the byproduct is now contaminated with some of the desired hexaether.

Approximately 28 g of partially purified product was dissolved in a minimum quantity of hexane and applied to a dry column containing 400 g of Baker chromatographic silica

gel below a section containing 100 g of Woelm dry column alumina in a 4.85 cm—diameter nylon column. The column was developed with the solvent 96.25% hexane—3.0% ethyl acetate—0.75% t-butanol. Approximately 400 ml of this solvent mixture is required to develop the column within about 3 cm of its bottom. At no time was the head of liquid above the column greater than 2 cm.

None of the components are ultraviolet absorbing, and puncture analysis alone was used to locate the individual components. Taking samples spaced every 2 cm, the product was found completely in fractions 8 to 12 and dioctadecyl ether was widely separated in fractions 34-45. Both these fractions were found in the silica portion of the column. Lower ethers were completely confined to the alumina portion and were also completely removed from the product. Elution of the product fraction after slicing the column, using ethyl acetate, gave 12.6 g of pure product. The resolution obtainable by this procedure is thus greater than that of common open column liquid chromatography, which in the past has never yielded this product in completely pure form.

Mannitol 1,2,5,6-tetraoctadecyl ether, 19. The entire product obtained in the previous step was dissolved in heptane (175 ml) containing 5 ml of glacial acetic acid. A catalyst was prepared by dissolving 1.0 g of palladium chloride in 5 ml of aqua regia, and after heating the solution for several hours on the steam bath as much acid as possible was removed in vacuo. The residue was taken up in water and about 2 g of sodium borohydride was gradually added to the mixture in an open beaker. The precipitated palladium black was filtered off on a sintered glass funnel without exposure to air, and washed with water, 99% ethanol, and finally heptane. The catalyst in heptane was washed into the reaction flask of the hydrogenator containing the hexaether solution. Hydrogenation at 50 lb/in^2 was carried out for 24 hours. Only 4 lb of hydrogen pressure decrease was noted, and the product was mostly starting material with only about 10% of tetraether formed. Another portion of catalyst prepared exactly as before from 1 g of palladium chloride was now added to the hydrogenation flask. Air was removed and hydrogenation was allowed to proceed, again initially at 50 lb/in^2. This time hydrogenation proceeded rapidly and was complete within 4 hours; TLC showed only tetraether. Filtration of the palladium catalyst and evaporation of the filtrate gave the product. Resolution of this residue in a small amount of ether followed by reprecipitation with acetonitrile gave the pure tetraether in quantitative yield. The palladium catalyst

10.59

was washed with heptane, then ethanol, then water and dissolved in nitric acid for future use.

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SECOND QUARTERLY REPORT: DAMD-17-76-C-6073

Contractor: Long Island Jewish-Hillside Medical Center

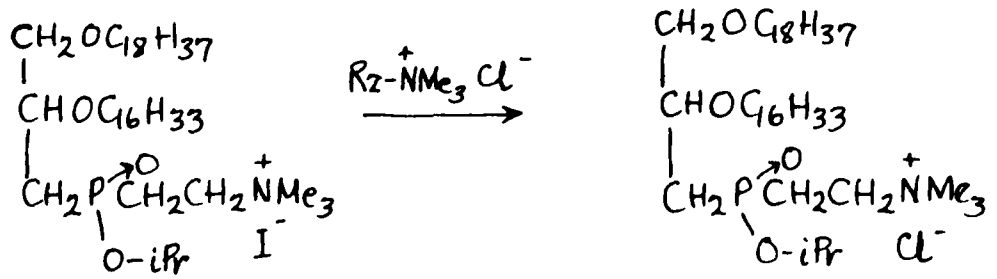
Principal Investigator: Dr. Arthur F. Rosenthal
Department of Laboratories
Long Island Jewish-Hillside Medical Center
New Hyde Park, New York 11040

212-470-2516

Period: September 23 - December 31, 1976

The objective of the contracted work continues to be the chemical synthesis of analogs of the intermediates of phospholipid metabolism for use as potential antimalarial agents.

At the conclusion of the first quarterly report, it was stated that work was continuing on the conversion of the following iodide into its corresponding chloride salt for purposes of providing the secondary target compound with a non-toxic anion:

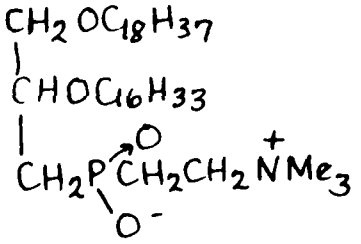


1 2

Actually, the difficulty which we had at that time was due to an erroneous analysis by our reference microanalytical laboratory, which at first stated that the compound submitted to them contained no chlorine. This caused us to repeat the preparation and reconsider the nature of the product formed by ion exchange. On reanalysis, however, the laboratory found approximately the correct value for chlorine, and a sample was subsequently submitted to WRAIR for antimalarial testing. The only real problem in the ion exchange is that, as is usual with lipids, a nonaqueous solvent had to be employed, since neither salt forms true solutions in water. This means that the resin (Amberlite IR-400 chloride) had first to be equilibrated overnight with the solvent before it was usable in the desired ion exchange.

The product was found to be unusually easily dispersible in water, and somewhat more polar in its solubility behavior than could be easily rationalized on the basis of its structure and by analogy with similar compounds. It is not very soluble in chloroform alone, but becomes completely soluble when methanol-chloroform mixtures are used. Probably the best route for testing would be as an aqueous dispersion obtained by sonication in water or glucose solution.

Although the object of preparing the secondary target chloride 2 was to provide additional cellular penetrability through the phosphinate ester, the zwitterionic lecithin analog 3 itself is the substance which is presently known to act as an inhibitor of phospholipase A in certain systems[1], and, therefore, was the major target compound in this series.



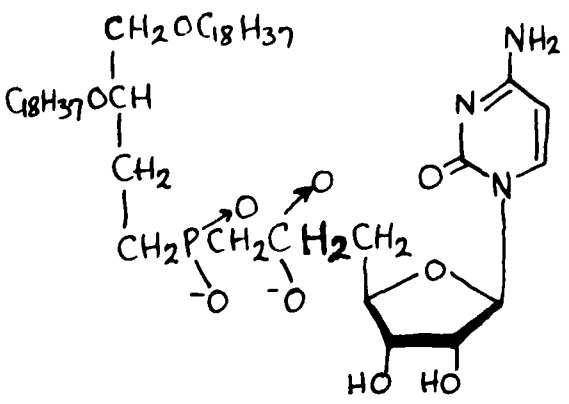
3

The theory behind the selection of this target compound, of course, is the apparent requirement of Plasmodium for a source of fatty acids from its host, due to the limited ability of the parasites to synthesize fatty acids *de novo*; and to the demonstrated hydrolysis of host plasma and red cell phospholipids to supply this requirement.

Hydrolysis of the ester in dilute hydrochloric acid in acetic acid was readily accomplished, but was accompanied by an interesting wrinkle not previously seen in this preparation. A small impurity was observed for the first time just trailing the main lecithin analog spot in the solvent system

usually employed (chloroform-methanol-water, 65:25:4). Removal of this impurity required, first of all, that a better solvent be found to produce a wider separation. After a number of trials, the following system was found to give satisfactory results: chloroform-methanol-acetic acid-water, 75:15:10:0.3. The wide separation thereby achieved was then transferred to column chromatography on Baker 3404 silica gel. An adequate removal of the small impurity was achieved thereby, but a new impurity, insoluble in chloroform and surprisingly difficult to remove from the product, was found. On investigation this was determined to be calcium acetate; the silicate gel apparently contained a small amount of calcium silicate, which released calcium in contact with the acetic acid of the solvent system used for elution. The calcium salt was finally removed by the use of Sephadex G-25, and 3 grams of pure lecithin analog 3 was sent to WRAIR.

Most of the remaining time during the second quarter of this contract was spent in preparing intermediates in large enough quantities to be useful in finally synthesizing target compounds related to the cytidine diphosphate diglyceride analogs below:



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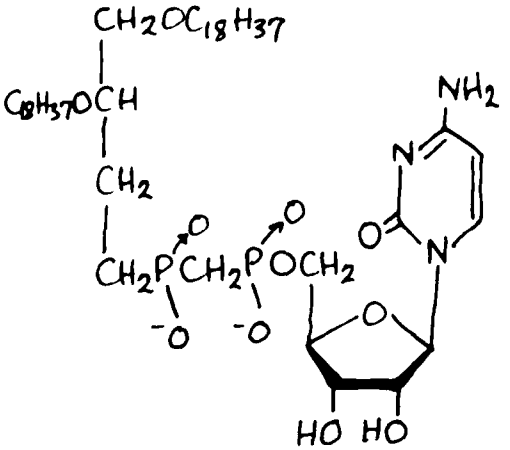

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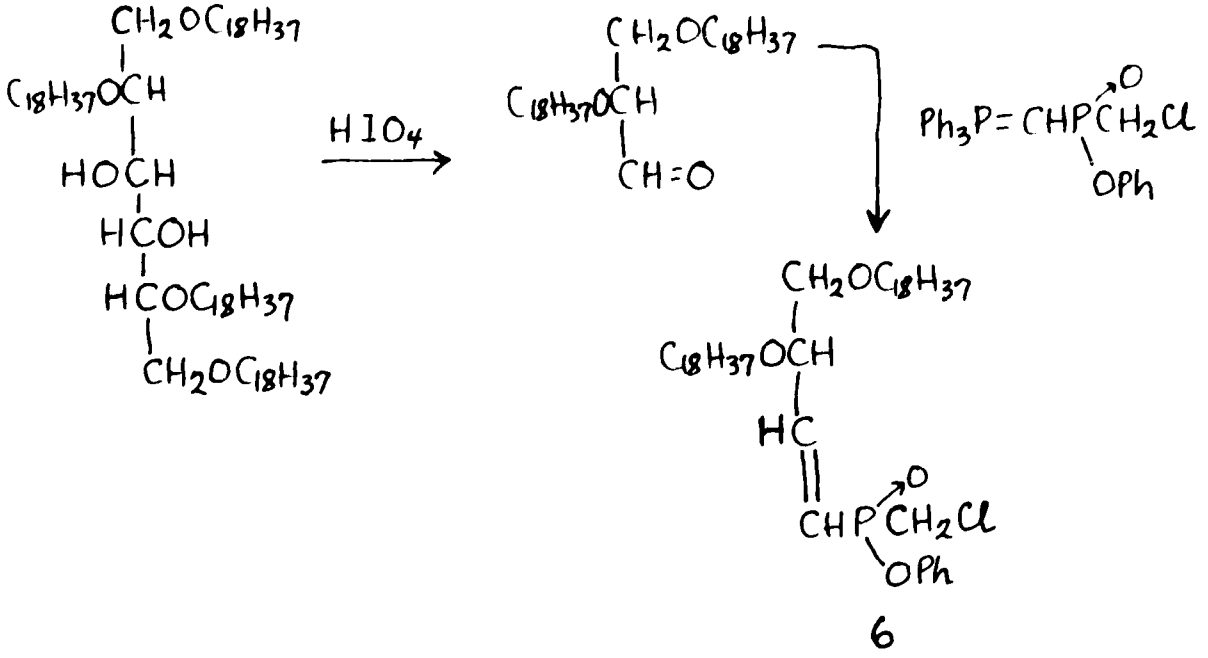
The reader is referred to schemes 1 and 2, pages 14 and 15, of the original contract application for a full exposition of the proposed syntheses of these compounds. Progress was made on both the lipid and nucleotide portions of the molecule, and intermediates containing both moieties were prepared.

Three separate preparations of D-mannitol 1,2,5,6-tetraoctadecyl ether, the immediate precursor of D-glyceraldehyde 2,3-dioctadecyl ether, were made. The first of these was actually accomplished during the first quarter and detailed description of the synthetic steps are found in the first quarter progress report for this contract. Subsequent preparations were essentially identical, and are, therefore, not described in detail herein.

D-Mannitol 1,2,5,6-tetraoctadecyl ether can be considered a stable storage precursor of glyceraldehyde 2,3-dioctadecyl ether, from which the latter may be easily generated by reaction with periodic acid. This was done on a fairly large scale and immediately reacted with the desired Wittig reagent to give the following reaction:



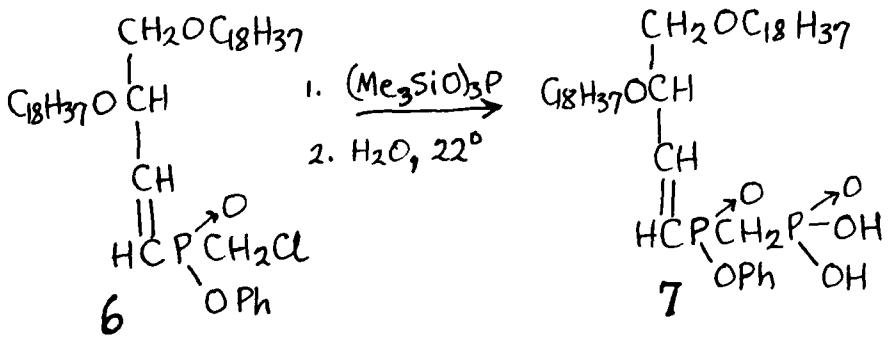
6

This reaction has been carried out previously (unpublished work). The formation of the glyceraldehyde diether has, however, been previously described [2]. The precursor phosphonium salt of the Wittig reagent has been fully characterized in the past (unpublished).

The reaction products are difficult to obtain in a desirable state of purity, probably because the proper fraction actually consists of a number of different products. Presumably, the Wittig reaction gives largely but not exclusively a trans product; both the cis and trans isomers exist in two diastereomeric forms, however (racemic about phosphorus but optically pure about carbon 3 of the side chain). Fortunately, all these isomers are desired products, since they will ultimately be converted into the same compound; but their presence at this stage of the synthesis creates considerable problems of purification. An additional problem which may be evident is the presence of a double bond activated by a phosphoryl group, which labilizes it to Michael addition and possibly polymerization reactions. These various effects have apparently combined in the particular run under discussion to produce a yield after chromatography much lower than that which has been formed in the reaction, as judged by TLC of the crude reaction product. From about 13 gm of mannitol tetraether was obtained only about 2 gm of chromatographically purified chloride 6 . It has been concluded that when this reaction is next run, which should be very early in the third quarter, no chromatographic purification will be attempted at this stage, but the entire crude product will be reacted with the silyl phosphite or phosphonite reagents.

The formation of the CDP-diglyceride analog 5

requires tris(trimethylsilyl)phosphite for the next step of the process:

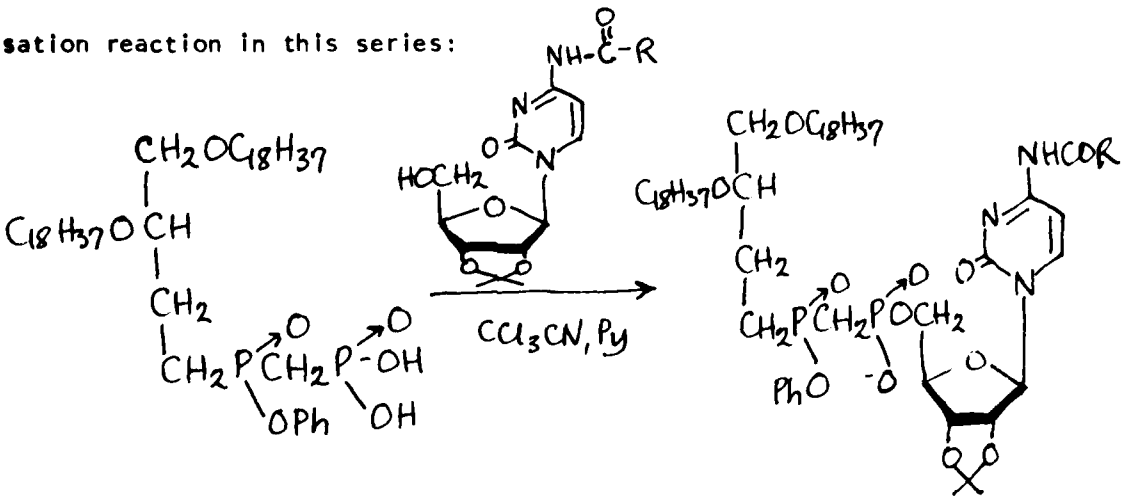


Previously, this valuable intermediate has been prepared by reaction of trimethylchlorosilane and triethylamine with phosphoric acid. This, of course, required the filtration of triethylamine hydrochloride before distillation of the product could be achieved; but tris(trimethylsilyl)phosphite, being air-labile, was partially decomposed during this process despite precautions. A much simpler preparation has been now conducted by the use of trimethylsilyldiethylamine. Preparation of this compound from trimethylchlorosilane and diethylamine also requires filtration of the amine hydrochloride, but in this case the desired product is only sensitive to the moisture and not to the oxygen of the air, so that much less decomposition occurs. Treatment of phosphorous acid with trimethylsilyldiethylamine was followed by simple fractional distillation of the product and byproducts, which had widely different boiling points. The desired phosphite was thus obtained in very good yield. At the conclusion of the second quarter, reaction of the chloride and tris(trimethylsilyl)phosphite was just being undertaken.

The cytidine portion of the molecule was also the object of considerable effort during this quarter. Prior studies indicated that not only the ribose 2',3'-hydroxyls require protection, but also the 4-amino group of the cytosine. Thus, two likely protected isopropylidene-cytidine derivatives were prepared, the N-phenoxyacetylamino and N-acetylamino compounds.

Using both of these protected derivatives, model condensations with 2-hexadecoxy-3-octadecoxypropyl phosphonic acid using trichloroacetonitrile in pyridine as condensing agent were investigated. No attempt was made to isolate the products because most of the required information could be obtained simply by performing a thin layer chromatographic study. This was made particularly easy by virtue of the fact that the starting phosphonic acid is not ultraviolet absorbing at around 260nm, while the cytidine derivative is; on the other hand, the cytidine derivative is not shown by molybdenum-molybdate spray, which visualizes phosphorus derivatives well[3]. Thus, condensation of the two starting materials can readily be observed by TLC, the desired product being indicated by a spot of approximately the expected polarity which is both ultraviolet absorbing and observable by the phosphorus-specific spray. Poor reactions (such as are found with unprotected cytidine derivatives) are indicated by many spots of product containing phosphorus and a cytidine moiety, or by lack of condensation (persistence of the starting materials). Conditions were soon found under which both the N-phenoxyacetylamino and N-acetylamino cytidine derivatives would undergo good condensation with the model phosphonic acid.

This highly encouraging result seemed to augur well for the key condensation reaction in this series:



EXPERIMENTAL

Only the preparation of intermediate or target compounds directly within each synthetic sequence is discussed in detail.

The preparation of 1 iodide is discussed in the previous progress report, as is the preparation of D-mannitol 1,2,5,6-tetraoctadecyl ether. Trimethylsilyldiethylamine is a commercial intermediate, but it is fairly expensive and, for these syntheses, was required on a scale large enough to make its synthesis in the laboratory economically desirable. It was prepared as follows: To a mixture of anhydrous diethylamine (927 ml) and 450 ml of pentane at -5° to -7° was added, with vigorous mechanical stirring, trimethylchlorosilane (572 ml) during 4.5 hours. The mixture was allowed to warm to 10° overnight without stirring (insulated bath). The mixture was filtered using positive nitrogen pressure through glass wool with three washes (total, ca. 3 l) of pentane, a process which occupied about 6 hours. The filtrate was fractionally distilled through a long Vigreux column wrapped with heating tape. Solvent was removed at $35 - 50^{\circ}$; after a small intermediate fraction, the product was collected at $123 - 127^{\circ}$; yield was about 600 ml. Purchased commercially, this amount of reagent would cost about $150.00, while the cost of reagents for preparing it is less than $20.00.

Isopropyl 2-hexadecoxy-3-octadecoxypropyl[2'-(trimethylammonium)ethyl] phosphinate, chloride salt, 2. The iodide 1 (11.5 g, 0.0132 mole) was converted to the chloride 2 by passing a solution of the latter in methanol chloroform 2:1 through a column of Amberlite IR-400 chloride (100 ml) previously equilibrated with the same solvent. The column was thoroughly eluted with the solvent and the combined eluates were evaporated to dryness. The product was recrystallized from methylene chloride-acetone to give 6.3 g (56 percent) of a white powder,

which sintered above 70° and liquified with decomposition at 163° - 167°. Another 2g of product was obtained from the filtrate. The product gave no precipitate with cupric acetate solution (negative test for iodide) and a positive Beilstein test. The sodium fusion test for chloride was slightly positive; it must be remembered, however, that the compound contains less than 5% chlorine. After some difficulty with the microanalytical reference laboratory, the following analysis was obtained for monohydrate: Calculated, C, 67.67%; H, 12.24%; N, 1.75%; P 3.88%; Cl, 4.44%. Found, C, 67.41%; H, 12.85%; N, 1.97%; P 3.89%; Cl, 4.69%.

2-Hexadecoxy-3-octadecoxypropyl [2'-(trimethylammonium)ethyl] phosphinic acid (inner salt), 3. The crude chloride 2 (5.5 g) was dissolved in 200 ml of acetic acid and to this solution was added 130 ml 6N hydrochloric acid. The turbid mixture was warmed to 70°- 75°, producing a clear solution; it was held at this temperature for 24 hours, at the end of which some turbidity reappeared. 50 ml more acetic acid was added to produce a clear solution once more and the mixture kept at 70° - 75° for 24 hours more. As much solvent as possible was removed in vacuo; repeated additions of isopropyl alcohol between repetitive evaporations greatly assisted in the removal of water. Final drying of the crude product yielded 4.9 g of material at this point.

This residue was dissolved in 20 ml of chloroform and 5 ml of pyridine; addition of a large excess of acetonitrile gave 4.7 g of product after filtration and drying. Since a small amount of material slightly more polar than the product was seen on TLC in chloroform-methanol-water, 65:25:4, a study was made to find the best solvent for separating this impurity from the desired product. On this basis, the following column chromatographic separation was performed:

The product (4.7 g) in chloroform was applied to a silica gel column (300 g, JT Baker 3404). Pure product was eluted with chloroform-methanol

- acetic acid-water, 75:15:10:0.3. The eluate fractions were monitored by TLC; in this experiment, using about 14 ml fractions, pure product was obtained in fractions 64-190. Fraction 40-90 contained pure product but contained a chloroform-insoluble byproduct which was found to be calcium acetate by infrared and atomic absorption analysis. Removal of this material was accomplished by passing a solution in chloroform-methanol, saturated with water, through Sephadex G-25 (7 g). From the two major fractions a total of 2.9 g of chromatographically pure product was obtained. This material has, of course, been previously characterized[4]. Repetition of the hydrolysis yielded sufficient material to send a total of 3.0 g to WRAIR for antimalarial testing.

Phenyl R-3,4-dioctadecoxybut-1-enyl(chloromethyl)phosphinate, 6. D-mannitol 1,2,5,6-tetraoctadecyl ether (13.1 g, 0.011 mol) was dissolved in 80 ml tetrahydrofuran and 500 ml of ether. Periodic acid (5.1 g, dried in vacuo) in tetrahydrofuran (40 ml) and ether (350 ml) was added during 15 minutes to the tetraether solution at 30° with vigorous stirring. A white precipitate (iodic acid) formed well before the addition was complete. Stirring was continued for two hours more at room temperature. The ether solution was decanted and the precipitate washed twice with ether. The combined ether layers were washed with water and then with 50 ml 5% sodium bicarbonate solution and then with water again. After drying the ether solution with magnesium sulfate and filtering, the solvent was evaporated thoroughly.

Phenyl chloromethyl [(trimethylphosphonium)methyl] phosphinate chloride (salt) (25.5 g) in 700 ml of water was warmed to give a clear solution. After cooling 300 ml of toluene was added and with vigorous stirring 50 ml of 20% aqueous potassium carbonate solution was added dropwise. The toluene layer was separated and the aqueous layer extracted with 50 ml of toluene and the combined

AD-A096 475 LONG ISLAND JEWISH-HILLSIDE MEDICAL CENTER NEW HYDE --ETC F/G 6/5
SYNTHETIC ANALOGS OF PHOSPHOLIPID METABOLITES AS ANTIMALARIALS.(U)
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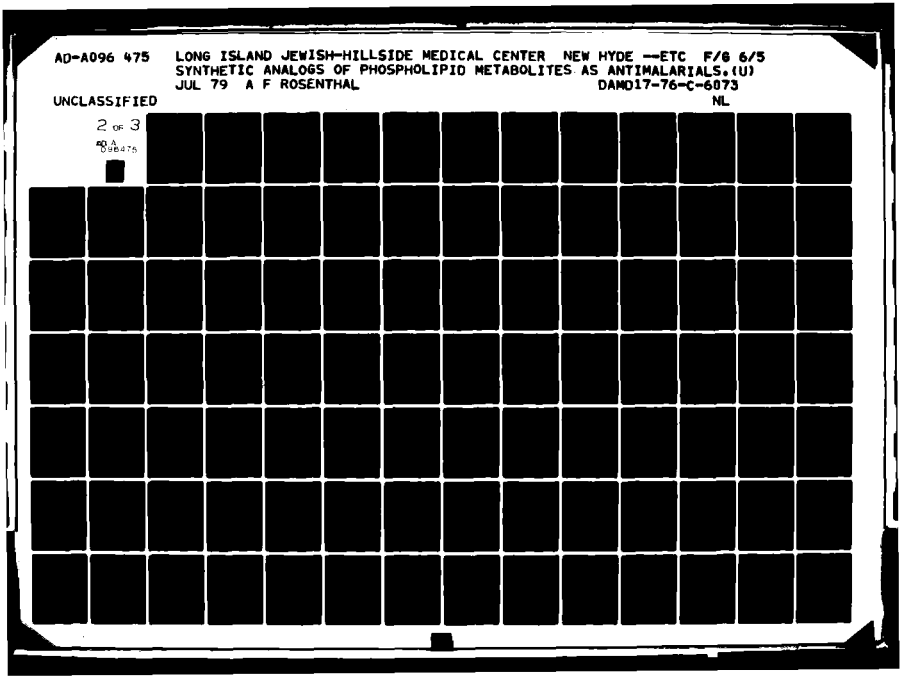

toluene layers washed with water and dried over anhydrous potassium carbonate. The filtered toluene solution of the ylide 5 was added to the residue from the periodate reaction and the clear yellowish solution was heated at reflux temperature for 72 hours.

The dark reaction mixture was separated from some toluene-insoluble oil and evaporated to dryness. The residue was precipitated with acetonitrile to give 15.7 g of crude product. The insoluble oil did not contain any material precipitable by acetonitrile.

Small scale attempts to separate the product by chromatography on Woelm dry column neutral silica did not yield useful separations. JT Baker silica (3404) gave a much better separation but poor recovery. Using a large scale separation less than 2 g was obtained from the 15 g of crude reaction product with 5% ethyl acetate in chloroform. The compound has, however, previously been characterized (unpublished work).

Tris (trimethylsilyl)phosphite. Phosphorous acid (41 g, 0.5 mol, previously dried in vacuo over P_2O_5) was dissolved in 60 ml of 1,2-dimethoxyethane (freshly distilled from calcium hydride). To this solution was added drop wise with good magnetic stirring and cooling in an ice-water bath 345 ml (1.8 mol) of trimethylsilyldiethylamine. The reaction is strongly exothermic and was accompanied by the transient appearance of a white solid which disappeared as the reaction continued. The addition occupied four hours, after which the mixture was heated to about 60° with stirring for two hours more and kept overnight at room temperature. Diethylamine was removed at atmospheric pressure between 56°and 75°; under water pump vacuum there was obtained 131 g (145 ml) of tris (trimethylsilyl)phosphite, bp_{22} 94°-96° (85%); reported[5], 86.5° at 18 mm.

2,4-Dinitrophenyl phenoxyacetate. The preparation of this compound is reported since no preparation could be found in the literature. 2,4-dinitrophenol (dried over 3205; 4.5 g, 0.0218 mol) was dissolved in 12 ml of anhydrous 1,2-dimethoxyethane and triethylamine (2.2 g, 0.0218 mol) was added. The deep orange solution was cooled to 0°- 5° and phenoxyacetyl chloride was added dropwise. A yellowish precipitate immediately formed and was filtered and washed with dimethoxyethane and ether; yield 76%;mp 95°-98°.

N-4-phenoxyacetyl isopropylidenecytidine was prepared from isopropylidene-cytidine and dinitrophenyl phenoxyacetate according to the procedure of Bleaney and Jones[6] in 40% yield; mp 177-178°. Infrared and nmr data agreed with those given by these authors. N-4-acetyl cytidine was prepared by the method of Watanabe and Fox[7]. Cytidine was reacted in methanol containing acetic anhydride to give a 72% yield of the product, mp 190° (decomp). The isopropylidene derivative was prepared by the method of Verheyden and Moffatt[9]; N-4-acetyl cytidine (2.4 g) was treated with 2,2-dimethoxypropane, acetone, and perchloric to give 2 g of the product.

Due to the high price of isopropylidene cytidine ($20 per g), a procedure for preparing this derivative from cytidine (about 20 per g) was investigated. Cytidine was reacted with 2,2-dimethoxypropane and dimethylformamide in the presence of hydrogen chloride according to the procedure of Chladek and Smrt[9]. It was found not necessary to purify the hydrochloride by ion exchange since it precipitated readily in 98% yield from the reaction mixture. Conversion of the hydrochloride to the free base was thus performed by passing it through Amberlite IR-400 (OH^-) in water according to the literature[10] to give the product as a glass.

Model condensations. 2-Hexadecoxy-3-octadecoxypropylphosphonic acid (0.1 m mol) and N-4-acetyl isopropylidenecytidine (0.2 m mol) in 2 ml of pyridine were treated with 0.2 ml of trichloroacetonitrile and the reaction mixture was stirred at 65° for 21 hours. The addition of cold acetonitrile to the reaction mixture gave a precipitate which was filtered out and washed with acetonitrile. In chloroform-methanol-water, 65: 25: 4, the following results were obtained: The phosphonic acid streak centered at Rf 0.62 had essentially disappeared in the reaction product and was replaced by a reasonably compact spot at 0.69 which was both phosphorus-positive and UV absorbing. The starting protected cytidine, Rf 0.76, was essentially absent from the reaction product; it was soluble in acetonitrile.

Employing the solvent system chloroform-methanol-formic acid (98%), 44.5: 5: 0.5, wider separation was observed between the starting protected cytidine derivative (0.49) and the condensation product (0.32), which now also showed a second smaller ultraviolet- and phosphorus-positive spot at 0.20.

Utilizing the same conditions for condensation with phenoxyacetylisopropylidene-cytidine, similar results were obtained. The phosphorus-negative cytidine derivative had Rf 0.83 and the condensation product had Rf 0.86, with small, slightly more polar, ultraviolet-absorbing impurities at 0.31 and 0.50. The use of the second solvent showed the main product of the condensation at 0.57 but with a new UV-absorbing but phosphorus-negative spot at 0.23. The phenoxyacetyl protecting group is known to be acid-labile, which could have accounted for this impurity in the second solvent system.

COMPOUNDS SUBMITTED

1) Isopropyl 2-hexadecoxy-3-octadecoxypropyl(allyl)phosphinate.

2) Isopropyl 2-hexadecoxy-3-octadecoxypropyl[2'-(trimethylammonium)ethyl] phosphinate chloride (salt).

3) 2-Hexadecoxy-3-octadecoxypropyl[2'-(trimethylammonium)ethyl] phosphinate.

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THIRD QUARTERLY REPORT: DAMD-17-76-C-6073

Contractor: Long Island Jewish-Hillside Medical Center

Principal Investigator: Dr. Arthur F. Rosenthal
Department of Laboratories
Long Island Jewish-Hillside Medical Center
New Hyde Park, New York 11040
(212) 470-2516

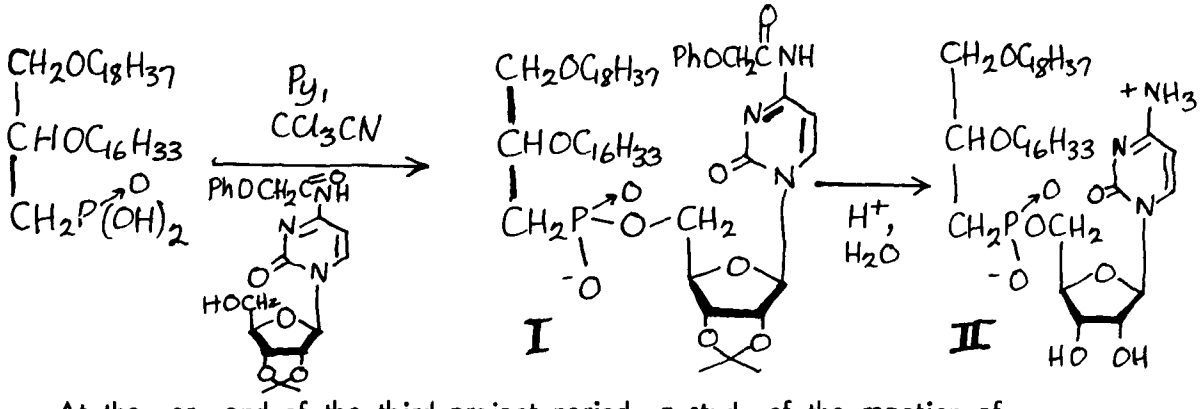
Period: January 1 — March 31, 1977

Toward the end of the second project period attempts, largely successful, to demonstrate by thin-layer chromatography the condensation between N-protected isopropylidenecytidine derivatives and a model lipid phosphonate were undertaken and reported in the Second Quarterly Progress Report (pp 6-7). In order to completely understand the characteristics of the condensation reactions, and to even more importantly obtain close to optimal conditions for the subsequent deprotection of the intermediate with liberation of the desired cytidine lipid phosphonate, and also to provide a potentially interesting model of a liponucleotide for antimalarial testing, a more intensive study was made of this series of reactions, this time with isolation of products.

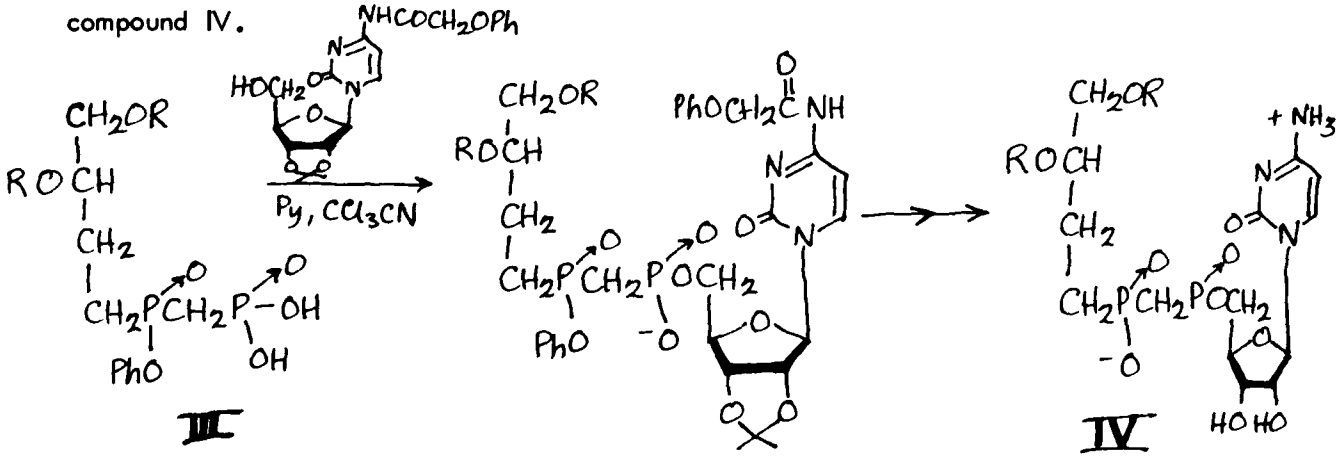
In condensations of this type, using either sulfonyl chlorides or trichloroacetonitrile as activating (condensing) agents, it has been usual [1,2] for a large excess of the alcoholic components to be employed. The reason for this is twofold: The reaction clearly proceeds to a greater degree of completion using an excess of one of the components, and in the preparations of phospholipids and their analogues from phosphatidic acid (or its analogues) and amino alcohols, it is almost always the latter which is by far the cheaper component; and it is generally much easier to separate the lipid product from the water soluble amino alcohol than it is to separate the final phospholipid from an excess of phosphatidic acid or its analogues. In our case, however, with this model reaction the alcoholic component (protected cytidine) is the more valuable component and in a condensation to produce a CDP-diglyceride analogue itself would be not very much less valuable than the acid component. Thus, it seems important to determine whether useful yields of condensation products could be obtained without using the large excess of alcohol, which in reactions of this type has often exceeded 1000 percent.

Reaction of 2-hexadecoxy-3-octadecoxypropylphosphonic acid in pyridine solution with only a 1 molar excess of N^4-phenoxyacetyl-2',3'-isopropylidenecytidine gives a moderately good yield of the protected condensation product I, which could be satisfactorily purified by recrystallization. Investigation of conditions

under which removal of the phenoxyacetyl and isopropylidene protecting groups could be effected without hydrolyzing the phosphonate ester indicated that deprotection was somewhat more difficult than anticipated. Nevertheless, aqueous trifluoroacetic acid completely removed the protecting groups with little or no detectable injury to the P-O-C bond. The model liponucleotide II was isolated from the deprotection reaction in excellent yields. After purification by recrystallization, the product was satisfactorily characterized by elemental analysis, IR, NMR and UV. Approximately 700 mg was submitted to WRAIR for testing.

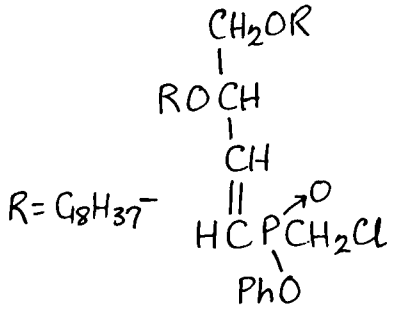


At the very end of the third project period, a study of the reaction of the phosphinic-phosphonic acid analogue III with the same protected cytidine derivative was just undertaken as the final step in the synthesis of the target compound IV.

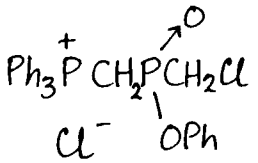


R = $C_{18}H_{37}$—

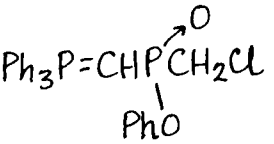
As is usual in syntheses which involve a good deal of research before a large scale final run can be made, one frequently runs out of intermediates and has to stop the research portion of the work in order to prepare the substances in short supply. In the previous progress reports, the difficulties of obtaining the key intermediate V were discussed. Unfortunately, our previous work also depleted our supply of the phosphonium salt intermediate VI.



V



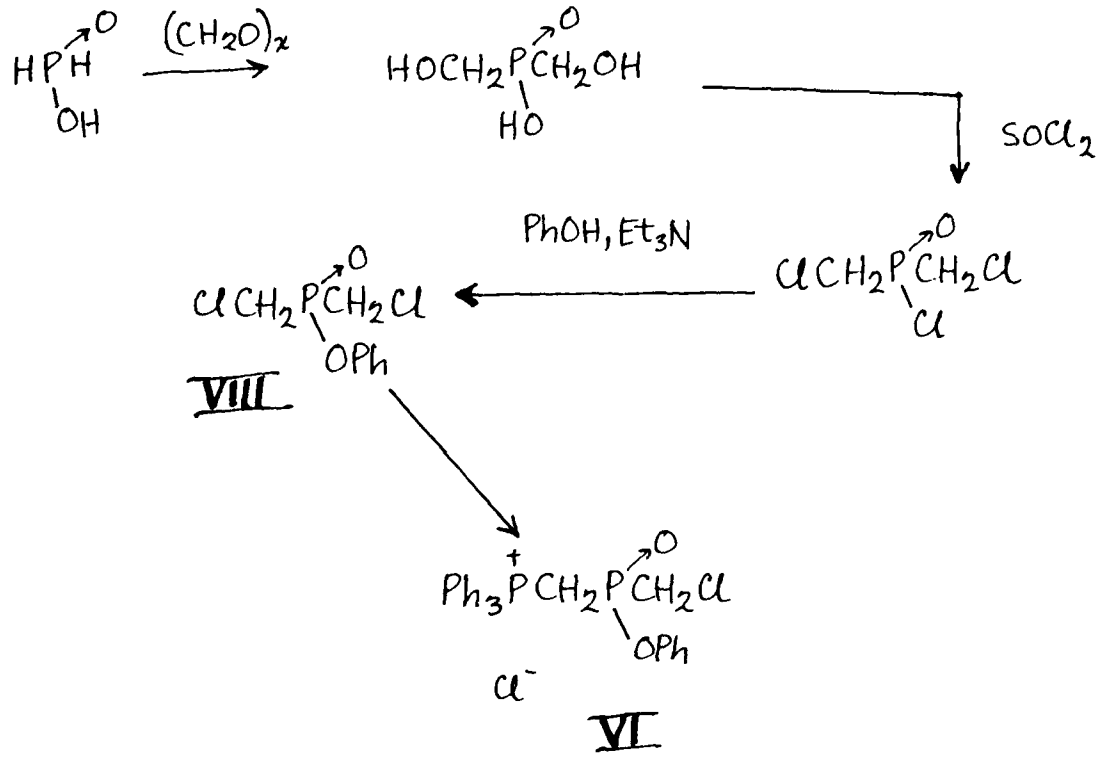
VI



VII

Thus, it became necessary to stop other work and to prepare this key substance. How far back in the synthetic sequence we had to go in order to do so is an interesting story, but the net effect was one of delaying the real business of this project. Such difficulties as we have encountered are fairly common and particularly felt in a project which, as we have indicated, is less than optimally manned.

The phosphonium salt, the usual precursor of the ylid VII (from which by reaction with glyceraldehyde diether the intermediate chloride V is prepared) is synthesized by quaternization of triphenylphosphine with phenyl bis(chloromethyl)phosphinate. This, in turn, is prepared from bis(chloromethyl)phosphinic chloride, which is itself prepared from bis(hydroxymethyl)-phosphinic acid. This acid, however, is no longer commercially available; the reason is quite interesting.



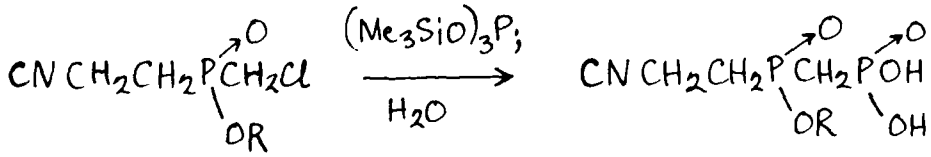
The usual preparation of this acid calls for reaction of sodium hypophosphite with an excess of paraformaldehyde and hydrochloric acid. As is now known, this combination of formaldehyde and HCl is most suspect, since even in the aqueous solution a certain amount of bis-chloromethyl ether, a potent carcinogen, is likely to form. It is unlikely that the market for bis(hydroxymethyl)phosphinic acid is sufficient to justify a commercial venture in finding alternative means for the manufacture of this acid, and the result has simply been to remove the phosphinic acid from the commercial market. Chloromethylphosphonic dichloride, an intermediate which we find very valuable as well, is also now apparently commercially unavailable for the same reason. Its manufacture involves reaction of paraformaldehyde with phosphorus trichloride, with consequent liberation of hydrogen chloride.

Since bis(hydroxymethyl)phosphinic acid was an essential intermediate in our synthesis, we had no alternative but to prepare it ourselves by a method which did not involve contact of formaldehyde with hydrochloric acid. This

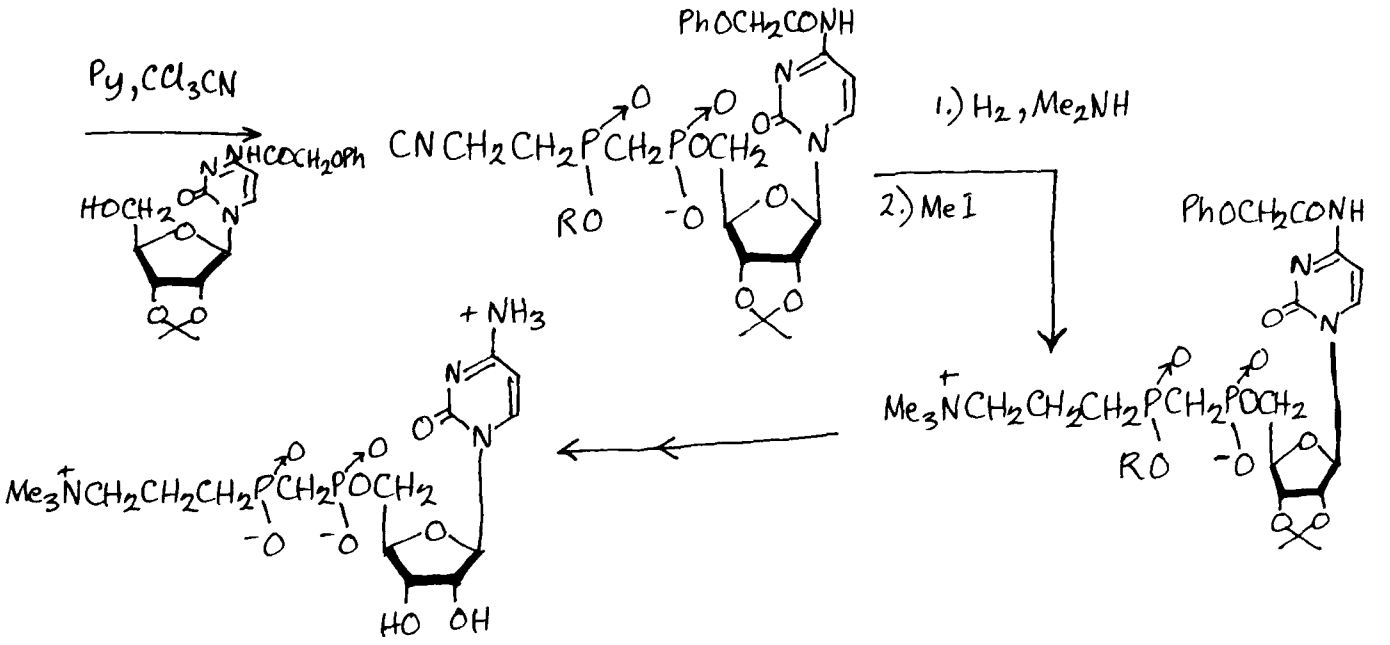
turned out to be not quite as simple as it seemed. Reaction of hypophosphorous acid with an excess of paraformaldehyde in aqueous solution at 60° overnight gave a product which was found ultimately not to be satisfactory for the preparation of bis(chloromethyl)phosphinic chloride, its phenyl ester, and the phosphonium salt VI. Some indirect evidence accumulated (see below) to the effect that this particular bottle of hypophosphorous acid may have been contaminated with appreciable phosphorous acid. Whatever the reason for the poor result, however, the problem was overcome by the use of a new bottle of hypophosphorous acid and/or the preparation of bis(hydroxymethyl)phosphinic acid under more vigorous conditions, as detailed in the Experimental section. From the phosphinic acid the trichloride, phenyl ester, and phosphonium salt VI were all prepared satisfactorily as in previous syntheses beginning with the commercially obtained hydroxymethylphosphinic acid.

From the ylid VII and D-glyceraldehyde dioctadecyl ether we obtained intermediate V without difficulty; the product was used, as projected, in a crude state. From this chloride was prepared the phosphinic-phosphonic acid analogue III, which had previously been characterized. Full details of this synthesis will be given in a later report. Briefly, the chloride is treated with tris(trimethylsilyl)phosphite and the free phosphonic acid liberated by treatment with water. The double bond was then hydrogenated to give the completely saturated acid and the condensation with protected cytidine undertaken (see above).

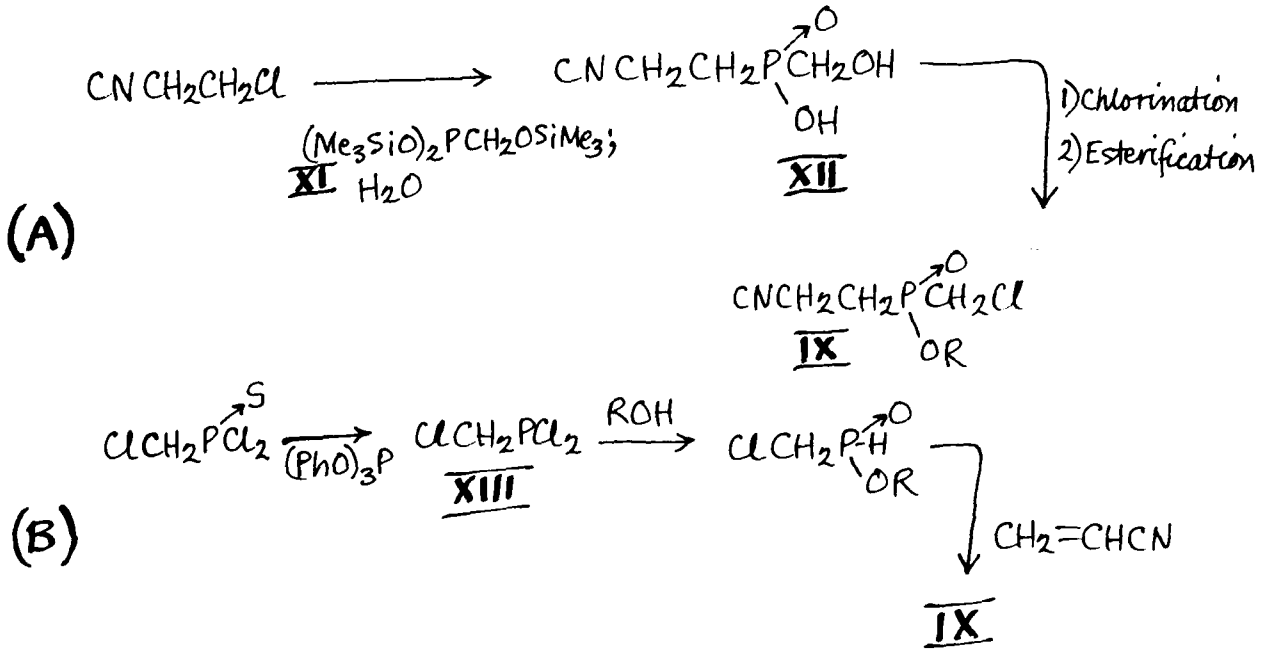
During this quarterly period reactions in the following synthetic sequence were also investigated:

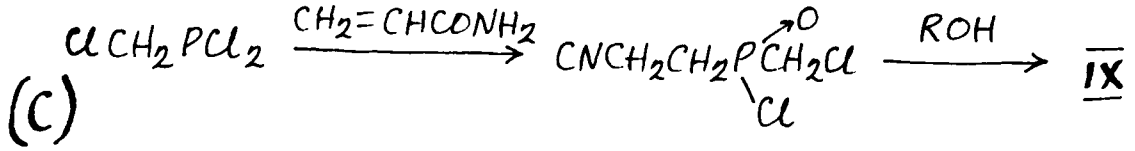


IX R = Ar or Alkyl



Three separate routes were considered for the formation of the important intermediate IX.





The initial steps in route A have already been explored in the past. The preparation of the phosphonite XI and of the acid XII has already been reported [3] but not described in detail. The silyl reagent XI is prepared by reaction of 1 mol of formaldehyde (as paraformaldehyde) with 1 mol of hypophosphorous acid; the reaction mixture is thoroughly dehydrated by azetropic distillation with isopropyl alcohol and the residual oil treated with trimethylsilyldiethylamine. The mixtures of completely silylated derivatives of hypophosphorous acid, hydroxymethylphosphonous acid (the desired silyl reagent), and bis(hydroxymethyl)phosphinic acid were readily separable by distillation. Redistillation of the product fraction gave the desired reagent XI in moderate yield.

This was found to undergo a normal Arbuzov reaction with 2-chloropropionitrile; treatment with water of the reaction product remaining after distillation of any remaining starting materials and trimethylsilylchloride gave hydroxymethyl(cyanoethyl)phosphinic acid, XII.

Considerable difficulty was observed in the past in preparing the chloro ester IX by direct chlorination followed by esterification. In a small scale study of this sequence the problem has been apparently solved in a very simple manner. The chlorination was performed by the use of oxalyl chloride and esterification accomplished by the use of phenol under reduced pressure (to remove the hydrogen chloride formed). In previous studies the use of pyridine and phenol or triethylamine and phenol or even diisopropylethylamine failed to give appreciable quantities of the expected product. Removal of excess phenol by sublimation following solvent partition yielded a product which on

gas chromatography (using both a carbon-sensitive and phosphorus—sensitive detector) showed a product consisting of two major components of which the more volatile was much the larger. Nmr showed a phenyl-to-aliphatic hydrogen ratio of 6:5; the theoretical ratio for the pure compound is 5:6.

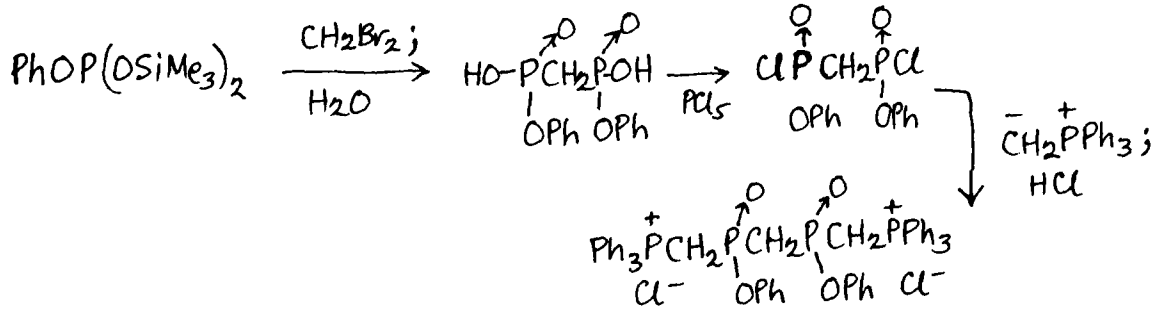
The constitution of both products was solved by mass spectroscopy. Chemical ionization first of all showed that the component in the earlier peak had a molecular weight of 243, while that of the later gas chromatographic peak was 287. The latter on electron impact showed a much larger fragment of m/e at 93, which could logically be ascribed to the phenoxy group. These facts alone showed that the 243 peak was the desired chloromethylphosphinate compound IX, while the 287 compound was diphenyl chloromethylphosphonate. The remainder of the fragmentation pattern was entirely consistent with these structural assignments.

Reasoning back to the beginning of the reaction sequence, one of the likely ways that diphenyl chloromethylphosphonate could have been formed was for the original hypophosphorous acid to have contained an appreciable quantity of phosphorous acid. Since the sample of the silyl reagent was an old one, one can also imagine more complex de-hydroxymethylation or de-trimethylsilyloxmethylation reactions having proceeded as well. The major piece of evidence not in accord with the presence of appreciable phosphorous acid in the original hypophosphorous acid is the fact that tris(trimethylsilyl)phosphite should have been easily removable from bis(trimethylsilyl) trimethylsilyloxmethylphosphonite, since the latter was distilled on a high efficiency column (the former silyl compound has a much lower boiling point). Thus, some more complex and less readily apparent mode of formation of tris(trimethylsilyl)phosphite from the silyl reagent XI may be a preferred explanation.

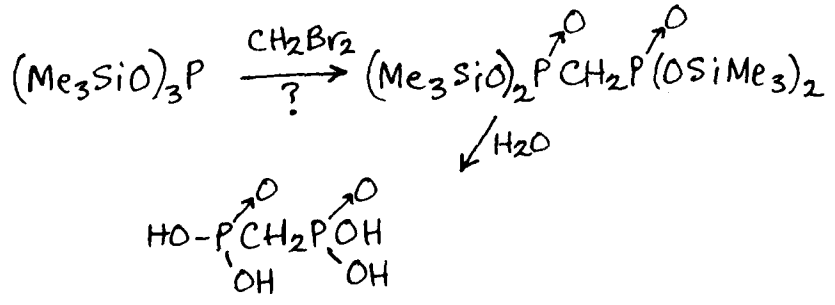
Despite the presence of the contaminant, the method was considered to have been a success in preparing the desired chloromethyl reagent IX. Concurrently, route B was studied toward the end of the project report period. The motivation for this was the apparently simpler preparation of chloromethylphosphonous dichloride XIII than of the silyl compound XI as the trivalent phosphorus reagent. Of course,

XIII cannot function as an Arbuzov reagent, but theoretically should be able to add to the activated double bond of acrylonitrile either by itself or through its monoalkyl ester. Monoethyl hydrogen chloromethylphosphonite was prepared from chloromethylphosphonous dichloride, which is very readily prepared in turn from chloromethylthiophosphonic dichloride. At the very end of the third project quarter possible Michael addition of the compound to acrylonitrile was under study. Preliminary work using a sodium hydroxide-tetrabutylammonium bisulfate catalyst had given only phosphorus-free products. Radical addition of the dichloride was under consideration but had not yet been attempted. Meanwhile, the peculiar acrylamide addition-dehydration reaction C was just being considered. Although it goes in only 20% yield, it has the distinct advantage that has actually been carried out[4] by Russian authors, and might serve to provide authentic intermediates.

Finally, the possibility of performing the following reaction was investigated



by employing the following model reaction, since tris(trimethylsilyl)phosphite is much more easily obtained (and was on hand) than is phenyl bis(trimethylsilyl)-phosphite:



The intent of this sequence was to prepare a bifunctional phosphonium salt which could be reacted separately at either end; see page 16 of current contract application.

In the model reaction the formation of a methylene diphosphonic acid intermediate was followed by means of the typical and characteristic 1:2:1 triplet observable from the methylene protons at 2-3 ppm in the nmr. Heating methylene bromide with a 50% excess of tris(trimethylsilyl)phosphite under nitrogen for 4 hours at 110° and then for 16 hours more at 128° gave a spontaneous liquid distilate containing trimethylsilyl bromide and some compounds containing P-H bonds. The residue was then distilled in vacuo to remove unreacted phosphite. Various higher boiling fractions were also obtained. On water hydrolysis at room temperature the highest-boiling fraction showed a small amount of a characteristic triplet. It was estimated that no more than 7% of the total material could be accounted for as methylene diphosphonic acid derivatives, and thus it was concluded that this type of reaction is unlikely to be preparatively useful.

EXPERIMENTAL

Cytidine 5'-(2"-hexadecoxy-3"-octadecoxypropyl)phosphonate, II. The preparation of phenoxyacetyl isopropylidenecytidine has been detailed in a previous progress report. The protective cytidine (1.38 g, 3.3 m mol) and 0.945 g (1.49 m mol) of 2-hexadecoxy-3-octadecoxypropylphosphonic acid were dissolved together in 21 ml of freshly distilled anhydrous pyridine. Six ml of trichloroacetonitrile was added and the mixture allowed to stand overnight at 53°. Pyridine was evaporated and ethanol containing a little water was added and the mixture was reevaporated to remove residual pyridine. Finally, the residue was taken up in a small amount of chloroform-ethanol and an excess of acetonitrile added and the product which precipitated was filtered.

The cream-colored solid was dissolved in chloroform-methanol-water (3:2:1) and washed four times with 30 ml portions of 0.05 M HCl, and finally with water. The chloroform layer was dried over magnesium sulfate and evaporated to give a pale yellow glass. This was triturated with 60 ml of 1:1 acetonitrile-methanol, filtered and dried in vacuo to give 1.1 g (72%) of crude product containing a small quantity of protected cytidine. The various mother liquors on evaporation were found to contain isopropylidene-phenoxyacetylcytidine and phenoxyacetylcytidine.

The entire crude product was dissolved in a mixture of 3 ml of trifluoroacetic acid, 0.7 ml of water and the solution kept at 50° for 24 hours. The solution was evaporated to dryness and reevaporated after addition of isopropyl alcohol; this procedure was repeated several times to completely remove water from the mixture. The residue was taken up in a small amount of chloroform, methanol and acetonitrile were added, and the white precipitate was filtered off. A small amount of additional white product could be obtained by evaporation of the mother liquor and reprecipitation.

The product was dissolved in chloroform and filtered to remove a small precipitate. The solvent was evaporated and ether was added; the ether suspension was filtered to give 0.85 g (94%) of the desired model liponucleotide. The material does not have a definite melting point: at 95-97° it sinters, becomes opaque about 107°, yellows at 140°, becomes amber at 150-160°, but remains opaque until 170°, when it clears but does not flow. After drying in a high vacuum at 80° the compound analyzed as an anhydrous zwitterionic form.

Calculated: C, 64.35; H, 10.34; N, 4.90; P, 3.61
Found: C, 64.60; H, 10.45; N, 4.63; P, 3.54

Ir showed the expected pattern; e.g., 3350-3050 cm^{-1} broad (OH); 1710 and 1650 cm^{-1} (ring CONH-); 1280 cm^{-1} (P→O); 1210-1170 cm^{-1} broad (H-bonded P→O); 1100 and 1055 cm^{-1} (ether). In the uv two absorption maxima were seen at 230 and 280 nm in a ratio of about 1:2·5 (cytidine moiety). The nmr in trifluoroacetic acid showed the absence of the aromatic ring but was otherwise of limited usefulness due to the broadness of the absorptions.

The phosphinic-phosphonic acid phenyl ester III was a sample prepared before the inception of the project. The preparation of the chloro compound which

serves as its immediate precursor was described on page 10 of the second quarterly report. Hydrogenation of the acid was performed in a warm solution of tetrahydrofuran-acetic acid, using a 10% Pd/C catalyst at 50 lb of hydrogen. Condensation of this saturated acid with isopropylidene-phenoxyacetylcytidine was just undertaken at the end of the project quarter. All these synthetic steps will be reported in detail at a later time.

Bis-(Hydroxymethyl)phosphinic acid. A mixture of 240 g of paraformaldehyde (8.0 mol equivalents of formaldehyde), 250 ml of 50% aqueous hypophosphorous acid (2.3 mol), and 3 ml of concentrated sulfuric acid were stirred under nitrogen in an oil bath at 105° for 42 hours. The mixture became clear after the first two hours of reaction. Water and other volatile material was removed as thoroughly as possible in vacuo, with a bath temperature below 62°. Residual water was removed by reevaporation a number of times with isopropyl alcohol. The final crude product was a very viscous, clear, faintly yellowish liquid.

The preparation of Bis-(chloromethyl)phosphinic chloride has been reported by Meier[5]. We found it very advantageous to carefully purify the product by fractional distillation through a long Vigreux column in order to obtain satisfactory products in subsequent steps. A fraction of $bp_{0.3}$ 79-85° was found to be of adequate purity.

The preparation of phenyl bis-(chloromethyl)phosphonate is also to be found in the literature[6]. By reaction of the phosphinic chloride with phenol and triethylamine, all in equivalent amounts, we obtained a 63% yield of the ester ester after distillation; $bp_{0.15}$ 134-136°. The pure compound crystallizes on standing.

Chloromethyl(phenoxyphosphino)methyltriphenylphosphonium chloride, VI. A solution of 100 g of triphenylphosphine (0.38 mol) in 800 ml anhydrous xylene was filtered to obtain a completely clear solution. To this was added 89.6 g (0.38 mol) of bis-(chloromethyl)phosphinic acid phenyl ester. After addition of 100 ml more xylene the mixture was heated at 110° for a total of 5 days to yield, in two crops, 106 g (56%) of the title compound VI, mp 235-236°. This product has previously been characterized by elemental analysis, ir, and nmr.

Bis(trimethylsilyl)trimethylsilyloxymethylphosphonite, XI. Although this compound was not prepared during the present project period, its synthesis is described because it was redistilled and used in work done during the first three months of 1977. To 42 ml of 50% aqueous hypophosphorous acid was added, with stirring under a nitrogen atmosphere, 11.5 g of paraformaldehyde portionwise during 2 hours at 40-45°. After 2 hours more the temperature was raised to 50° and the mixture was stirred overnight. Both reactants are present in 0.38 mol quantities; the crude acid was not separated from by-products, and its yield could not be estimated at this step.

The reaction mixture was evaporated as thoroughly as possible in vacuo (bath, below 50°). Isopropyl alcohol was added and the mixture reevaporated. This step was repeated many times to remove as much residual water as possible.

The clear oily liquid was mixed with 50 ml of anhydrous acetonitrile. Under a nitrogen atmosphere 285 ml (1.5 mol) of trimethylsilyldiethylamine was added dropwise. The rate of addition was controlled to prevent refluxing of diethylamine. The addition of silylamine consumed 4 hours.

The mixture was allowed to stir overnight at room temperature and then heated for 2 hours with stirring under reflux. The whole mixture was fractionally distilled. At water pump pressure diethylamine came off as a gas and excess trimethylsilyldiethylamine (ca. 100 ml) distilled at 45-55°.

The remaining mixture was then distilled under high vacuum using a long Vigreux column. The fraction $b_{0.025}$ 60-80° was taken as the crude product. An earlier fraction containing mostly silylated hypophosphorous acid and a later fraction containing mainly silylated bis(hydroxymethyl)phosphinic acid could also be isolated, but both contained only minimal amounts of desired product. The silylated hypophosphorous acid fraction is spontaneously inflammable in air. The same is true to a much lesser degree of the product fraction; this property is only shown if small drops are exposed to the atmosphere or particularly if droplets fall through air.

The product fraction was then redistilled, using a Nester-Faust spinning band annular teflon apparatus. It was not found possible to produce an adequate product

through any less efficient still. Fortunately, the purity of the product could be very well followed by the presence of only one type of methylene in the nmr, and its presence in the theoretical ratio to the two types of silyl protons. The hypophosphorous acid by-product shows the characteristic large P-H splitting and its presence could be thereby detected as well. The compound gives a satisfactory analysis for C, H, P, and Si. The boiling point at 0.5 mm is 51-53°; yield, 46%. Other constants are: d_{20}, 0.9092 g/ml; $n^{D}_{23.5}$, 1.4226 (nitrogen atmosphere).

2-Cyanoethyl(hydroxymethyl)phosphinic acid,XII. A mixture of 34 ml (0.10 mol) of the silyl reagent XI and 7.8 ml (8.4 g, 0.10 mol) of 2-chloropropionitrile were mixed under a very slow stream of nitrogen and immersed into an oil bath at 130°. After about 1/2 hour distillation of trimethylsilyl chloride began; the reaction was allowed to proceed at this temperature for 2 1/2 hours, at which time about 12 ml of the chloride had collected in the distilling receiver. After removal of low-boiling substances as much residual starting materials as possible were removed in high vacuum; only about 1 ml of material distilled. To the oily residue was added water (10 ml) and 1,2-dimethoxyethane (10 ml) and the mixture was stirred for 2 hours at room temperature. Water and solvent were removed as thoroughly as possible in vacuo; additional water was repeatedly evaporated with isopropyl alcohol. After a final thorough evaporation in vacuo (bath below 30°), the residual oil was swirled with hexane to remove liquid impurities and the mixture kept for several days at -5°. The crystals which formed were filtered off and dried in vacuo; the yield of product at this point was 12.8 g (86%). The product was recrystallized from acetone-hexane; the substance was a white crystalline material of mp 67-68°. It had previously been characterized by elemental analysis, infrared, and nmr.

Chlorination and esterification of this acid was accomplished as follows: to 750 mg (0.005 mol) of XII was added oxalyl chloride (5 ml), producing a vigorous evolution of gas. A trace of pyridine was added, coloring the entire mixture yellow. Additionally, a trace of dimethylformamide was added, and the mixture heated under reflux for 1 hour, during which it gradually became dark.

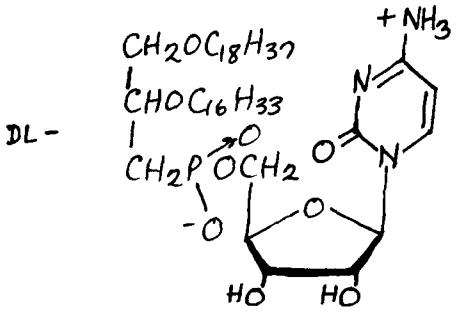
Excess oxalyl chloride was removed in vacuo and 1.0 g phenol (previously dried in vacuo) was added, producing a slow gas evolution. The mixture liquefied on heating and was kept at 70° for 24 hours in a vacuum of about 170 mm. The black reaction product was cooled, dissolved in alcohol-free chloroform, and extracted with saturated sodium bicarbonate solution, which produced no gas evolution. The mixture was stirred overnight and re-extracted three times with sodium bicarbonate solution and once with sodium sulfate solution. The very dark organic phase was shaken with activated charcoal overnight. Decolorization was mostly effective, but some color remained.

The charcoal was removed by filtration several times and the solution was dried over magnesium sulfate and evaporated in vacuo. The residual oil was subjected to high vacuum sublimation to remove phenol as thoroughly as possible (very little was liberated from the mixture).

Gas chromatography on OV-17 (using a 2-minute post-injection time followed by an 8°/min program to 255°) gave 2 major peaks at 10.0 and 14.1 minutes, of which the former was much the larger. Both peaks were observable by flame ionization and phosphorus thermionic detection. Mass spectroscopy indicated the larger peak to be the desired compound XII and the smaller to be diphenyl cyanoethylphosphonate, a conclusion which also accorded with the nmr data (see text above).

COMPOUNDS SUBMITTED

(1) Cytidine 5'-(2"-hexadecoxy-3"-octadecoxypropyl)phosphonate



(2) D-Mannitol 1, 2, 5, 6-tetraoctadecyl ether.

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Synthetic Analogs of Phospholipid Metabolites as Antimalarials

Annual Progress Report

(for the period 1 July 1976 - 30 June 1977)

By

Arthur F. Rosenthal, Ph.D.

September 28, 1977

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New Hyde Park, New York 11040

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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Phospholipid analogs. Lecithin analogs. Phosphatide antimetabolites. Liponucleotide analogs. Cytidine diphosphate diglyceride analogs. Cytidine diphosphate choline and cytidine diphosphate ethanolamine analogs and antimetabolites. Phosphonic acid analogs. Isosteric phosphinic acid analogs. Non-hydrolyzable antimetabolites.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Initial year's progress is described toward the synthesis of analogs of phospholipids and of their biosynthetic intermediates as possible antimalarials of a new type. Emphasis was placed on phosphonic and phosphinic acid analogs of lecithin, phosphatidic acid, cytidine diphosphate diglyceride, and cytidine diphosphate choline. Nine compounds were submitted of which one was a major target and three were secondary target compounds. None of the substances so far tested showed appreciable antimalarial activity.

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PERIOD: July 1, 1976 - June 30, 1977

1. Introduction.

This annual report describes our progress in synthesizing potential antimalarials of a novel type, which are designed to interfere with Plasmodial phospholipid metabolism. Very few prior attempts to produce antimalarial substances with this mode of action have been recorded, although a few previously known inhibitors of certain aspects of lipid metabolism (e.g., clofibrate) have been tested for antimalarial activity.

In part this almost complete neglect of Plasmodial phospholipid metabolism as a point on which to focus the design of new drugs is the result of paucity of substances which are known to interfere in any way, or with any specificity, with phospholipid metabolism in general. In addition, the crucial importance of phospholipid formation to the growth and reproduction of Plasmodia within its host has only begun to be appreciated very recently.

A much more detailed discussion of the biochemical rationale for this approach to the synthesis of new antimalarial drugs may be found in the original contract application. In the present report, the relevant metabolic steps will be mentioned only in passing. Furthermore, only those portions of the overall synthetic program which relate to progress during the first contract year will be discussed.

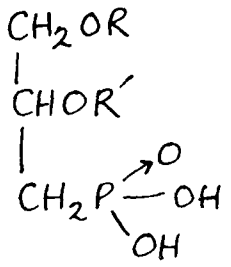
2. Synthetic Targets.

Each of the target compounds is an analog of a biosynthetic intermediate in phospholipid metabolism, either on the biosynthetic or catabolic side. Like their natural counterparts, these analogs can be produced with any of a variety of long-chain (R) groups, resulting in substances which may possess rather widely divergent physicochemical properties. This is particularly true in the case where two homologs differ in degree of unsaturation; this structural

feature is likely to result in more pronounced differences than are found in simple chain length homologs. The choice of R groups in the absence of medicinal activity data a priori must be arbitrary, and therefore other considerations have usually been predominant. Particularly, the question of probable technical simplicity in each synthetic step has generally dictated that in the lipid-containing analogs C_{16} and/or C_{18} saturated alkyl groups have been used. The generally ready crystallizability of such compounds makes the work-up following a synthetic step likely to be much simpler than would be the case with, e.g., polyunsaturated analogs. Should lead data suggest interesting medicinal activity of any of these substances, of course, new syntheses substituting various other R groups can be undertaken.

2.1 Phosphatidic Acid Analogs.

Phosphatidic acids occupy a key role in both the biosynthesis of glycerides and the formation of phospholipids. Diether phosphonate analogs of the following type



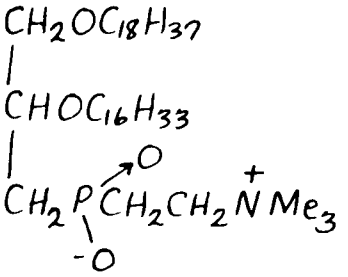
I

have been already shown (1,2) to be inhibitory towards phosphatidic acid phosphatase, a key enzyme in [illegible]-diglyceride formation. The synthetic scheme had already been worked out sometime previously (3), but for purposes of antimalarial testing, a new homolog, the di-C_{16}, was prepared. The synthetic steps (which were actually completed before the inception of the current contract) are shown

in Figure 1 and are discussed in considerable detail in the first quarterly contract report. Although the phosphonic acid proved to be without appreciable antimalarial activity, it is possible that it may be worthwhile to prepare a homolog with very different physicochemical properties (e.g., the dioleyl), since the activity of both these analogs and the natural substrates of phosphatidic acid phosphatase are highly dependent on physicochemical factors (1,2).

2.2. Lecithin Analogs.

Insofar as phospholipases can be inferred to be necessary for the phospholipid turnover of Plasmodial membranes, it seems reasonable to prepare a lecithin analog which has previously been shown to exert appreciable anti-phospholipase A (venom enzyme) and also anti-phospholipase C (clostridial enzyme) activity. This substance is a completely non-hydrolyzable analog of lecithin containing ether and phosphonate moieties instead of the normally labile carboxylic and phosphoric acid groups.



II

The synthesis of this compound has already been reported (4), so that it was necessary only to prepare the substance again on a scale large enough for extensive testing. In addition, its immediate synthetic precursor, the isopropyl ester chloride salt

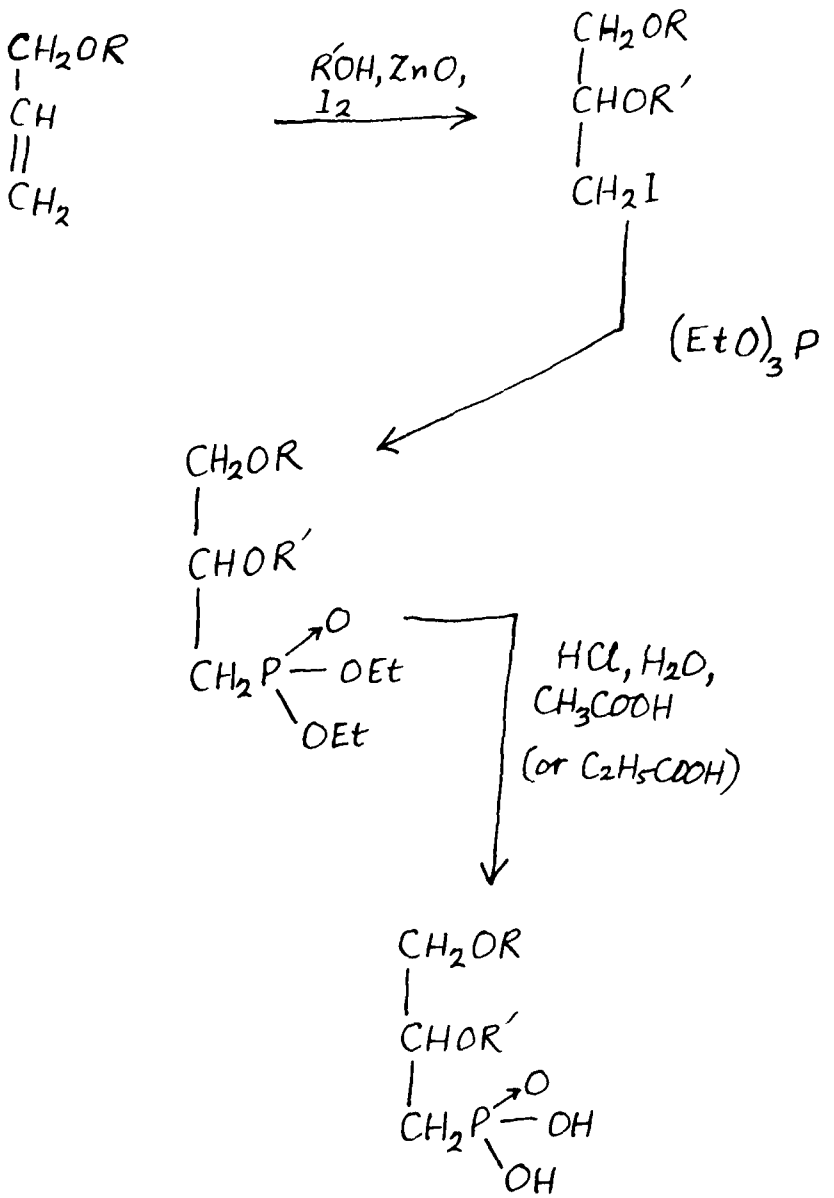
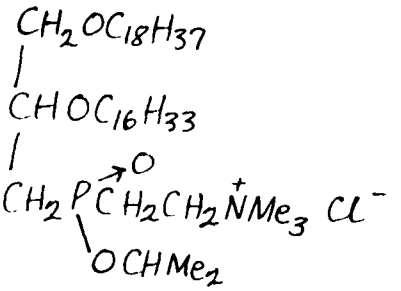

CH2OR
CH
CH2
R'OH, ZnO,
I2
CH2OR
CHOR'
CH2I
(EtO)3P
CH2OR
CHOR'
CH2P(O)(OEt)(OEt)
HCl, H2O,
CH3COOH
(or C2H5COOH)
CH2OR
CHOR'
CH2P(O)(OH)(OH)

FIG 1

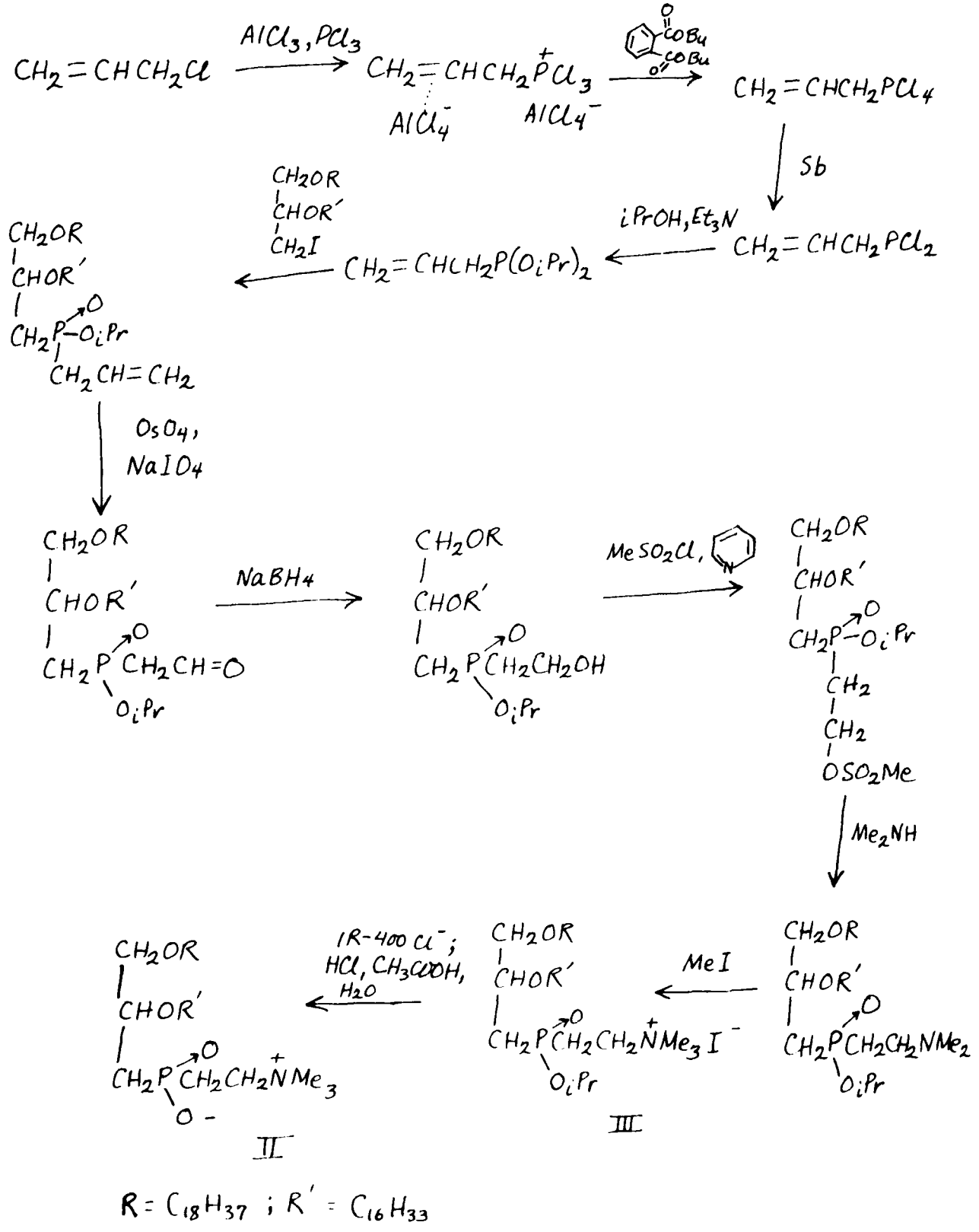


III

was taken as a secondary target compound. Its lesser ionic charge was believed to offer some additional hope of increased intracellular penetrability.

The synthetic routes to these compounds is found in Figure 2. The major point of difficulty in the entire synthesis is at the beginning, in the preparation of diisopropyl allylphosphonite. This reactive, air-sensitive intermediate is prepared through a succession of other labile intermediates and must be used in the Arbuzov reaction at once, since it is not capable of storage for any length of time. Reaction with 2-hexadecoxy-3-octadecoxyiodopropane gave the two diastereomeric forms of isopropyl 2-hexadecoxy-3-octadecoxypropyl(allyl)phosphinate, which were separated from impurities by dry column chromatography on silica gel.

Osmate-periodate oxidation followed by borohydride reduction gave 2-hydroxyethyl phophinate; mesylation yielded the mesylate ester satisfactorily. Reaction with dimethylamine produced the 2-dimethylaminoethyl phosphinate. Quaternization with methyl iodide gave the isopropyl ester of the quaternary ammonium phosphinate as the iodide salt. The insolubility of this substance in cold ether provides a very convenient means of purifying it from ether-soluble contaminants. If direct quaternization of the mesylate by trimethylamine is attempted, only dehydromesylation results.



$R = C_{18}H_{37}$; $R' = C_{16}H_{33}$

FIG 2

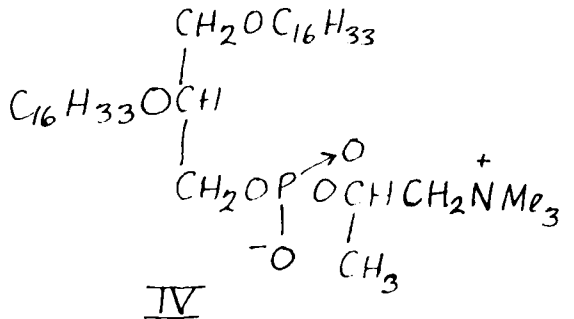
From the iodide salt the secondary target chloride III was readily prepared by ion exchange, using Amberlite IR-400 chloride in 2:1 methanol-chloroform. This salt had not previously been characterized and was purified by recrystallization for analysis.

Hydrolysis of the isopropyl ester chloride salt by hydrochloric acid in acetic acid gave the main target lecithin analog II, which of course had previously been characterized.

Full details of each step of this synthetic sequence are given in the first and second quarterly progress reports, and in reference (4).

Approximately 3.0 g of target compound 2 was submitted to WRAIR for testing. It was found to be inactive as an antimalarial agent. Enough of the compound was available for anti-Leishmanniasis testing A certain amount of suppression noted, but not of sufficient degree to be considered an active agent. It is possible that other homologs might later be prepared which would show enhanced activity in this respect.

During the fourth quarter, a lecithin analog of rather different type was prepared and sent for antimalarial testing. Although no biochemical information was available on this compound, it is somewhat more similar to a natural lecithin than the phosphinate analog described above. It differs primarily in having an unusual, branched-chain base in place of choline.



IV

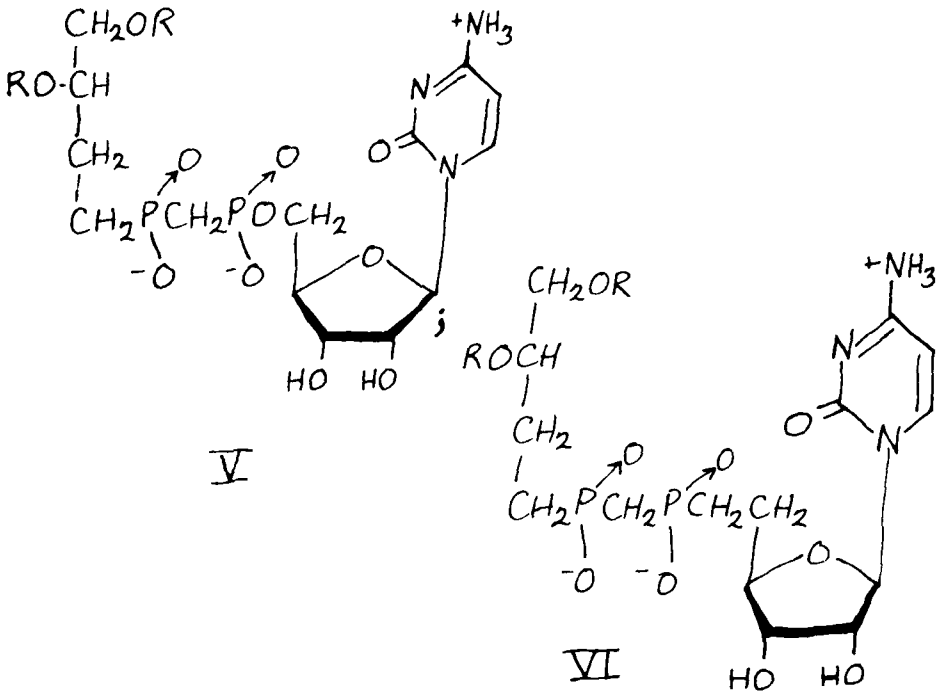
This synthesis was performed in a standard fashion. 1,2-dihexadecyl-sn-glycerol was prepared via D-mannitol, and phosphorylated with diphenylphosphoryl chloride. The protective phenyl ester moieties were removed by hydrogenation at room temperature and atmospheric pressure using a platinum catalyst. After isolation of the phosphatidic acid it was monoesterified by the tosylate salt of 2-hydroxy-1-(trimethylamino)propane, using trichloroacetonitrile in pyridine as the condensing agent. Precipitation by excess acetonitrile of the residue remaining after removal of volatile material gave a dark product. Passage through Amberlite MB-3 in tetrahydrofuran-water decolorized the product, which was finally purified by elution from a silica column. Final removal of silicic acid fines was accomplished by passage through a cellulose membrane filter. The material analyzed satisfactorily for a monohydrate form. More than 500 mg was submitted to WRAIR for antimalarial testing. This was too recent for us to have received a report as yet.

2.3. Analogs of Cytidine Diphosphate Diglyceride

Cytidine diphosphate diglycerides are a family of unique liponucleotides which are obligatory intermediates in the biosynthesis of phosphatidylserine,

with possible decarboxylation to phosphatidylethanolamine, of phosphatidyl-inositol, phosphatidylglycerol, diphosphatidylglycerol and several other less common phospholipids. (The only exception to the previous statement is the fact that in some organisms deoxycytidine diphosphate diglyceride can substitute for the corresponding ribose liponucleotides; The requirement for cytidine molety is essentially absolute, however.)

The liponucleotide analogs which are the ultimate targets of these synthetic efforts are the following:



V

VI

The much greater complexity of these substances requires that by far the greatest portion of our synthetic efforts go into this portion of the work, and that directed toward the synthesis of the

cytidine diphosphate choline and cytidine diphosphate ethanolamine analogs discussed below (2.4). Moreover, these synthesis involve a number of relatively unexplored areas of organic phosphorus synthetic chemistry, in which analogous known cases are non-existent. Therefore, a significant effort must be expended to find the most suitable way to perform a particular synthetic step. Thus, progress in this area is always somewhat slower than it might appear a priori.

Figure 3 shows the portion of the synthetic scheme which is common to both types of liponucleotide analog. Figure 3a illustrates the remaining steps in the synthesis of analog V while 3b shows the steps leading to analog VI. The R groups in these particular homologs are octadecyl; the fact that the synthesis begins with D-mannitol assures that the final product will be in the same optical configuration as the natural liponucleotides.

The preparation of mannitol 1,2,5,6-tetraoctadecyl ether has been discussed in the first quarterly progress report. A new technique, phase-transfer etherification, was used in place of the more common heterogeneous Williamson reaction to produce both D-mannitol 3,4-di-p-methylbenzyl ether and D-mannitol 1,2,5,6 tetraoctadecyl 3,4-di-p-methylbenzyl hexaether.

1,2,5,6 Diisopropylidene-D-mannitol was treated with an excess of p-methylbenzylchloride in diisopropyl ether solution. Aqueous potassium hydroxide and the tetrabutylammonium bisulfate catalyst were added and the mixture stirred below the boiling point of the solvent. Work-up afforded the diether in good yield after hydrolysis of isopropylidene groups. Treatment of this product with an excess of octadecyl bromide in a mixture of isopropyl ether and tetrahydropyran afforded the hexaether, which after preliminary purification was isolated by dry column chromatography

on a hybrid silica-alumina column.

Hydrogenation of the hexaether was readily accomplished, using a borohydride-reduced paladium catalyst. Full experimental details of this portion of the synthesis are given in the first quarterly report. Purified samples of the tetraether and hexaether intermediates were submitted to WRAIR for testing. To date no reports have been received for either substance.

Reaction with periodic acid in tetrahydrofuran gave D-glyceraldehyde dioctadecyl ether, which was immediately reacted with phenyl chloromethyl-(triphenylphosphinemethylene)phosphinate to give phenyl R-3,4-dioctadecoxybut-1-enyl(chloromethyl)phosphinate. Some considerable difficulty was found in purifying this chloromethyllipid by silicic acid chromatography, so that ultimately it was used in succeeding steps without complete purification. Experimental details are given in the second quarterly report, along with additional discussion of the problems of purification. Further reaction necessitated the preparation of additional amounts of certain intermediates. In fact, a large portion of the second and third quarters was concerned with building up stocks of particular intermediates which were required for further synthetic steps.

Tris(trimethylsilyl)phosphite had previously been prepared from trimethylsilyl chloride, triethylamine, and phosphorous acid; however, it became evident that the filtration step which was necessary in this preparation to remove triethylamine hydrochloride would make the procedure very cumbersome for large scale preparations. Thus, it was found that warming phosphorous acid in acetonitrile solution with trimethylsilyldiethylamine was a superior means of preparing the silylphosphite, and in fact produced higher yields. The silylamine, in turn, had to be prepared on a large scale in order to prepare the phosphite, and also bis(trimethylsilyl)trimethylsilyoxymethylphosphonite.

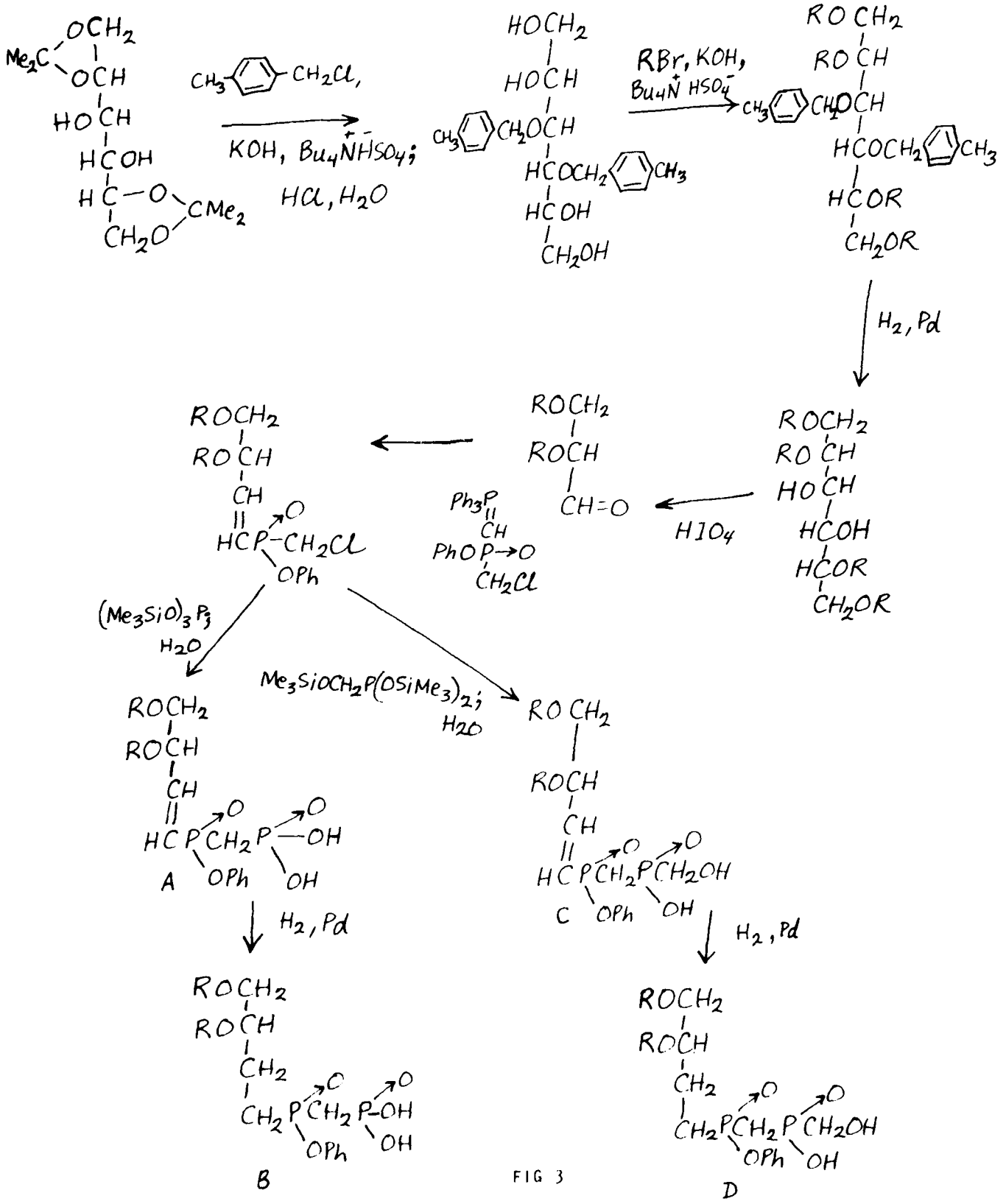
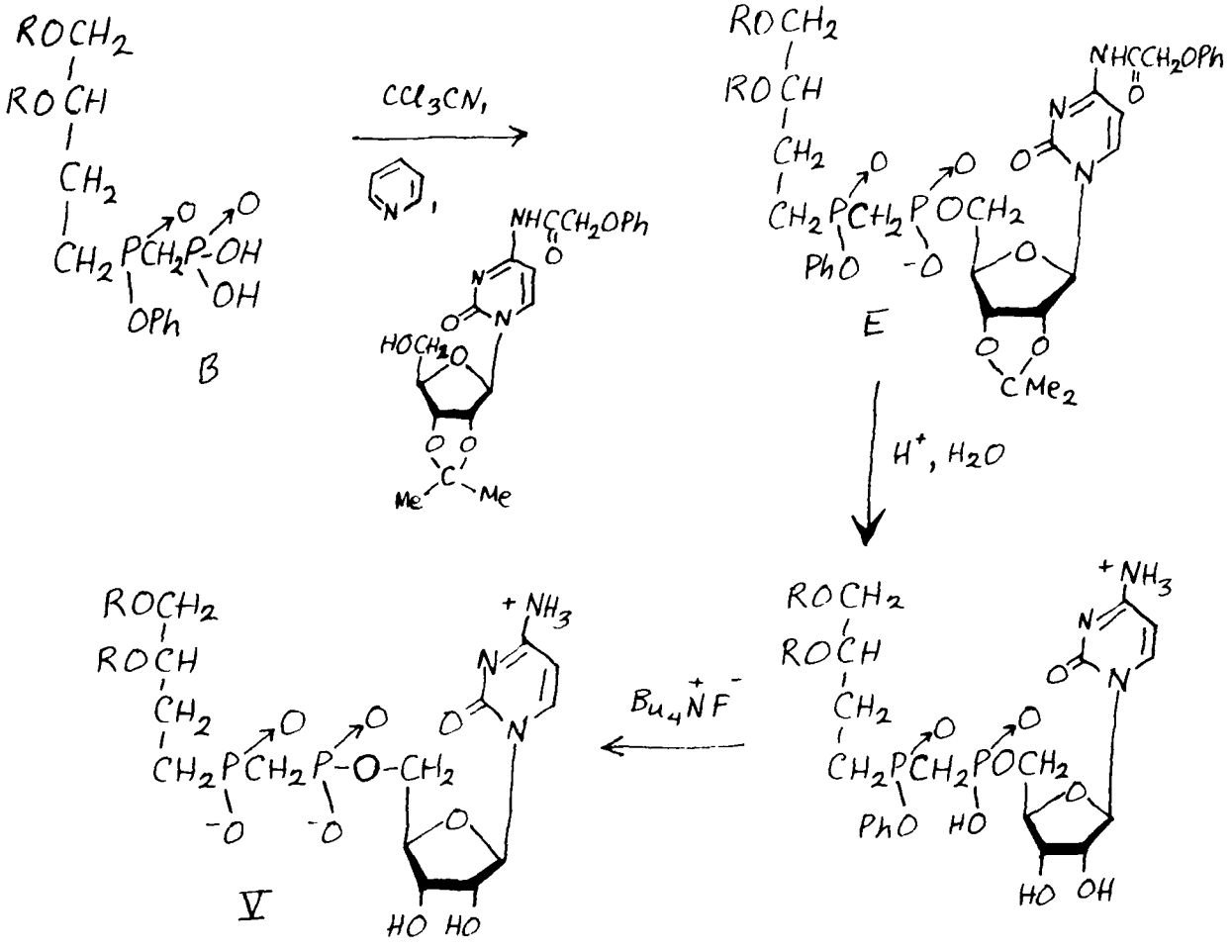
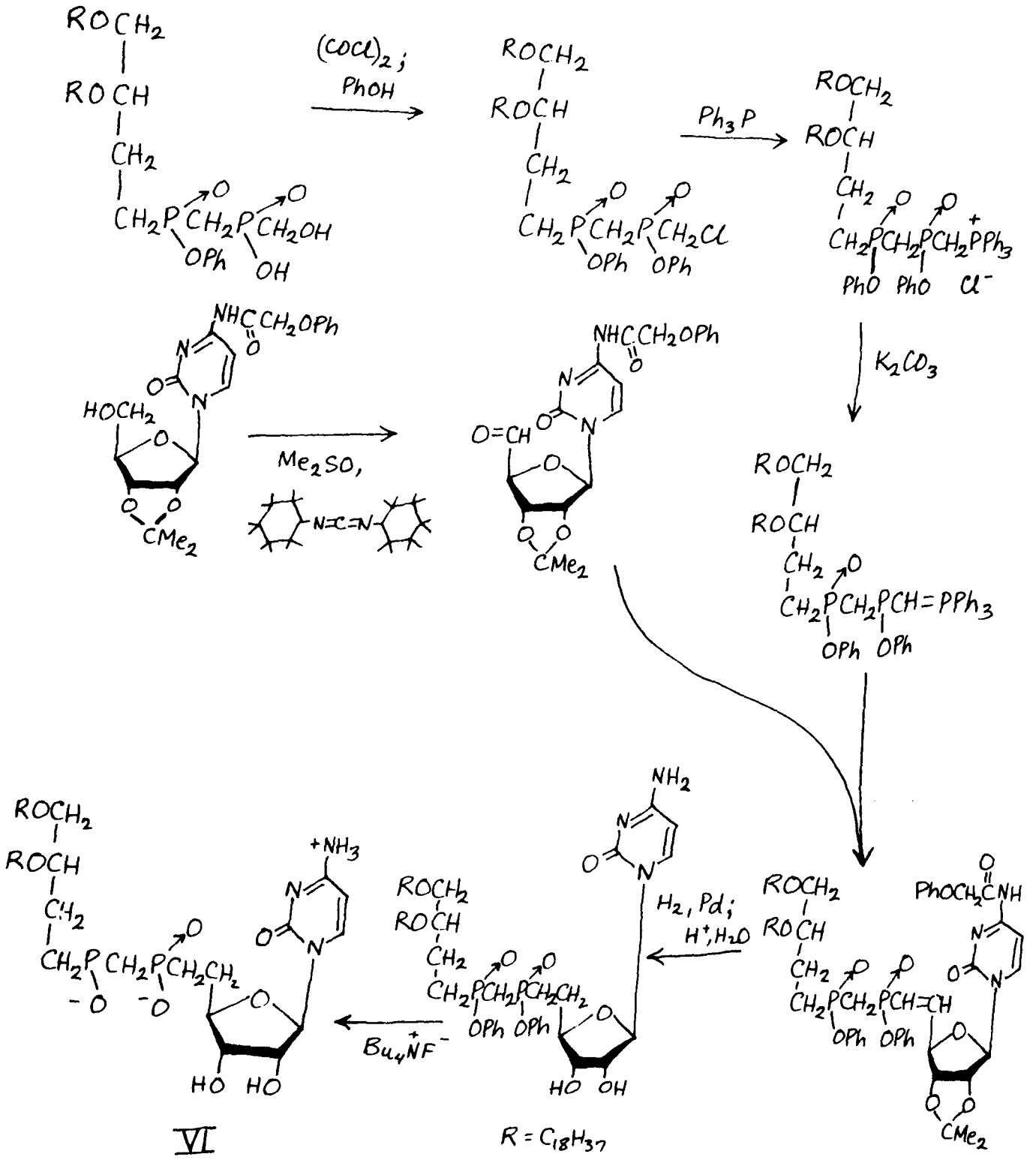

KOH, Bu4NHSO4; HCl, H2O
RBr, KOH, Bu4N+ HSO4-
H2, Pd
HIO4
(Me3SiO)3P; H2O
Me3SiOCH2P(OSiMe3)2; H2O
A
B
C
D

FIG 3



R = $C_{18}H_{37}$

FIG 3a



VI

FIG 3b

A large-scale preparation of the silylamine was therefore undertaken; the reactants are trimethylsilylchloride and diethylamine. The large amount of diethylamine hydrochloride which is produced must also be filtered off, which is quite tedious on a very large scale, but is preferable at this step since the product is not readily oxidizable in air as are the phosphite or phosphonite.

From the silylamine was prepared tris(trimethylsilyl)phosphite and bis(trimethylsilyl)trimethylsilyoxymethylphosphonite, and both were used to carry out further steps. An additional quantity of phosphinic-phosphonic acid phenylester was prepared for condensation with protected cytidine derivatives to explore the synthesis of analog V.

This analog and the corresponding analog of cytidine diphosphatecholine required a suitable quantity of a protected cytidine derivative. For this purpose N-phenoxyacetyl isopropylidene cytidine was prepared from 2,4-dinitrophenyl phenoxyacetate (in turn prepared from phenoxyacetyl chloride and 2,4-dinitrophenol) and isopropylidene cytidine. N-acetyl isopropylidenecytidine was also prepared from acetic anhydride and isopropylidenecytidine. These preparations are detailed in the second quarterly report.

In order to work out conditions for the condensation of the phosphonic acid with the cytidine derivatives to produce analog V, a thin-layer chromatographic study was first undertaken. Since the phosphinic-phosphonic acid was, of course,difficult to prepare, the conditions were worked out using a model long-chain phosphonic acid, 2-hexadecoxy-3-octadecoxypropylphosphonic acid. As was evident from the TLC studies, the condensation would occur under relatively mild conditions in the presence of trichloroacetonitrile. Deprotection of the resulting condensed product was more difficult, but ultimately was achieved.

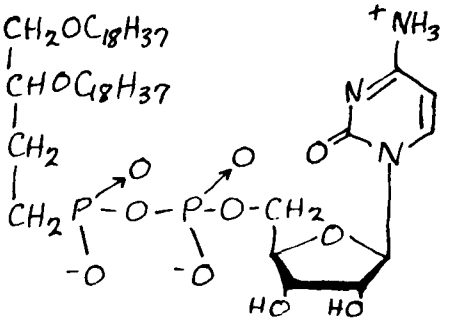
It then became possible to prepare a much larger quantity of the condensed model compound, and to purify it and send it to WRAIR for testing. More than 500 mg of cytidine 5'-2"-hexadecoxy-3"-octadecoxypropylphosphonate was sent; antimalarial testing data indicated that it was inactive. This may not be a surprising finding in view of the fact that, unlike the natural coenzyme, it contains no central P-O-P or its isostere.

The successful preparation of the model liponucleotide naturally indicated that the actual phosphinic-phosphonic acid compound B (figure 3) should be employed. Unfortunately, only a small amount of this substance was available and a thin-layer chromatographic study appeared the only feasible method of investigating this condensation reaction for the present. The preparation of much larger amounts of this acid, which should allow isolation and direct characterization of intermediates, must await the preparation of more tris-(trimethylsilyl)phosphite, which in turn is awaiting the large-scale preparation of trimethylsilyl diethylamine. The preparation of additional N-phenoxyacetyl isopropylidenecytidine is also being awaited for preparation of the final compound on sufficient scale.

In any event, it was quickly found that reaction of the acid B with the protected cytidine required more vigorous conditions than was the case of the model compound. It is possible that steric or other factors retard the condensation; when it was carried out at 50° overnight, very little condensed product (presumably E, figure 3a) was formed. However, raising the temperature to 70° while maintaining the reaction period and other conditions (pyridine solution, trichloroacetonitrile as condensing agent) showed that a reasonable yield of E was formed. A preliminary purification by precipitation with cold acetonitrile gave a product which retained a small amount of the protected cytidine, plus some polar, presumably acidic material,

but was otherwise mainly the desired product. Identification, as in the previous TLC studies with the model condensation, were made according to the spots which were UV absorbing (at about 260 nm) and contained phosphorus (Dittmer-Lester reagent). Only the condensation product should fulfill both criteria. The Rf values in chloroform-methanol-water-acetic acid (80:13:8:0.3) and in chloroform-methanol-formic acid (44.5:5:0.5) approximated those which would be expected from a protected liponucleotide of the expected degree of polarity.

Hydrolysis of the acid-labile protecting group was carried out in trifluoroacetic acid containing a small amount of water for 24 hours at 45°. Evaporation of volatile material, followed by solution of the residue in chloroform, filtration, re-evaporation, and finally precipitation by cold ether from a concentrated chloroform solution, yielded a product which appeared to be almost homogeneous by TLC. Since its Rf in the former solvent (above) was 0.6 compared to 0.58 for the following liponucleotide analog:



it is at present uncertain whether the trifluoroacetic acid treatment removed the protective phosphinic phenyl ester group. It was anticipated that a fluoride treatment would be necessary to remove this moiety, and it seems doubtful that the acid treatment would hydrolyze the phenyl ester without removing the cytidine ester group as well. Definitive characterization has not as yet been

possible. Infrared indicates no clear phenyl-type absorption, but these would occur in regions where other expected absorptions are found. The milligram quantities of materials so far available have not allowed nmr, which would have been definitive, to be performed. The large molecular weight also has militated against the possibility of obtained useful mass spectra, even if the compounds were derivatized. Therefore, a final answer must await the preparation of larger samples, for which we have some of the intermediates, but not others, on a sufficient scale as yet.

The synthesis and characterization of the bis-phosphinic acid derivative C and its dihydro derivative D were also the subjects of considerable effort during the fourth quarterly period. Reaction of crude phenyl 3,4-dioctadecoxybut-3-enyl(chloromethyl)phosphinate with bis(trimethylsily)trimethylsilyoxymethyl phosphonite on a small scale gave, after removal of excess phosphonite and other volatile material, followed by hydrolysis, a compound in reasonably homogeneous form which showed chromatographic, spectral, and solubility properties expected of the bis-phosphinic acid monophenyl ester. Definitive characterization of this substance was underway as the first quarter ended.

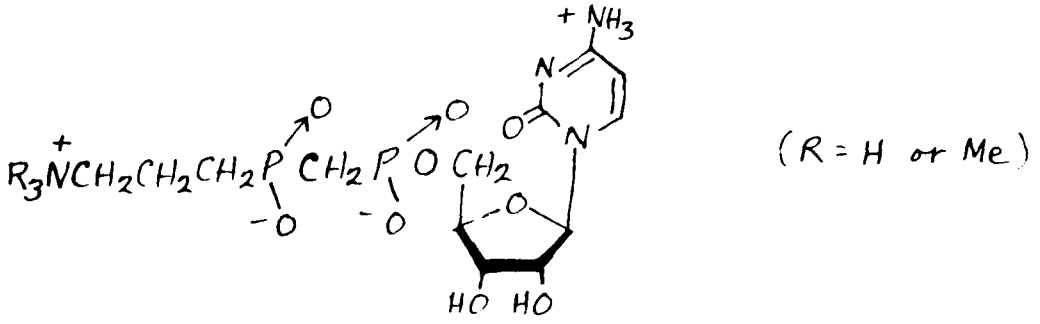
Hydrogenation of this product was also carried out, using 10 percent paladium on charcoal in a hydrogen atmosphere of 60 lb/in^2. Characterization of this product (D, figure 3) was also in progress as well.

2.4. Analogs of Cytidine Diphosphate Choline and Cytidine Diphosphate Ethanolamine.

The non-liponucleotide coenzymes cytidine diphosphate choline and -ethanolamine utilized 1,2-diglyceride rather than phosphatidic acid as the co-reactant, to produce lecithin and phosphatidyl ethanolamine directly. The coenzyme thus acts as a phosphorylated base donor rather than as a phosphatidyl donor,

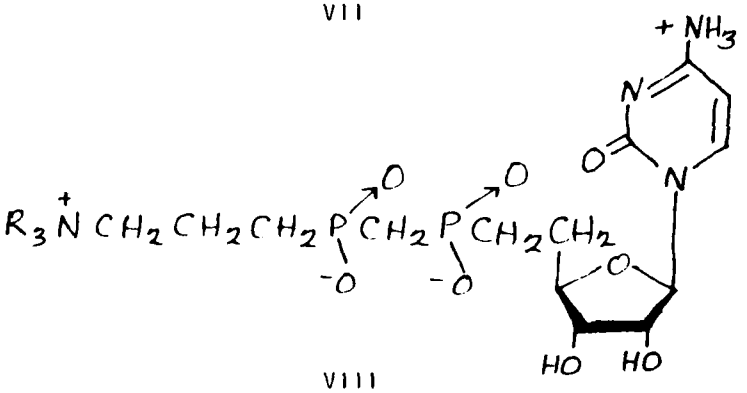
as in the case of CDP-diglyceride. This route is probably the major one for the formation of lecithin at least, and interference with the reaction should exert a significant effect on membrane phospholipid formation.

The target analogs of the base-dinucleotides have the same relationship to their natural coenzymes as do the liponucleotide analogs discussed above, and have the following structure:



(R = H or Me)

VII



VIII

The synthetic route to these compounds, however, is rather different from that discussed in 2.3 above. This is occasioned by the obvious difference between the base moiety and the diglyceride analog moiety. However, it should be carefully noted that certain features of the synthesis of compounds VII and VIII will serve as model reactions for analogous synthetic steps in the liponucleotide analog syntheses. The reverse, of course, is also the case. The synthetic schemes are shown in figures 4a and 4b. The preparation of the initial intermediate F of figure 4 has been the object of a great deal of investigation during the latter phases of this first annual project. Three separate and distinct synthetic routes were considered; these are shown in figure 5. Figure 5a

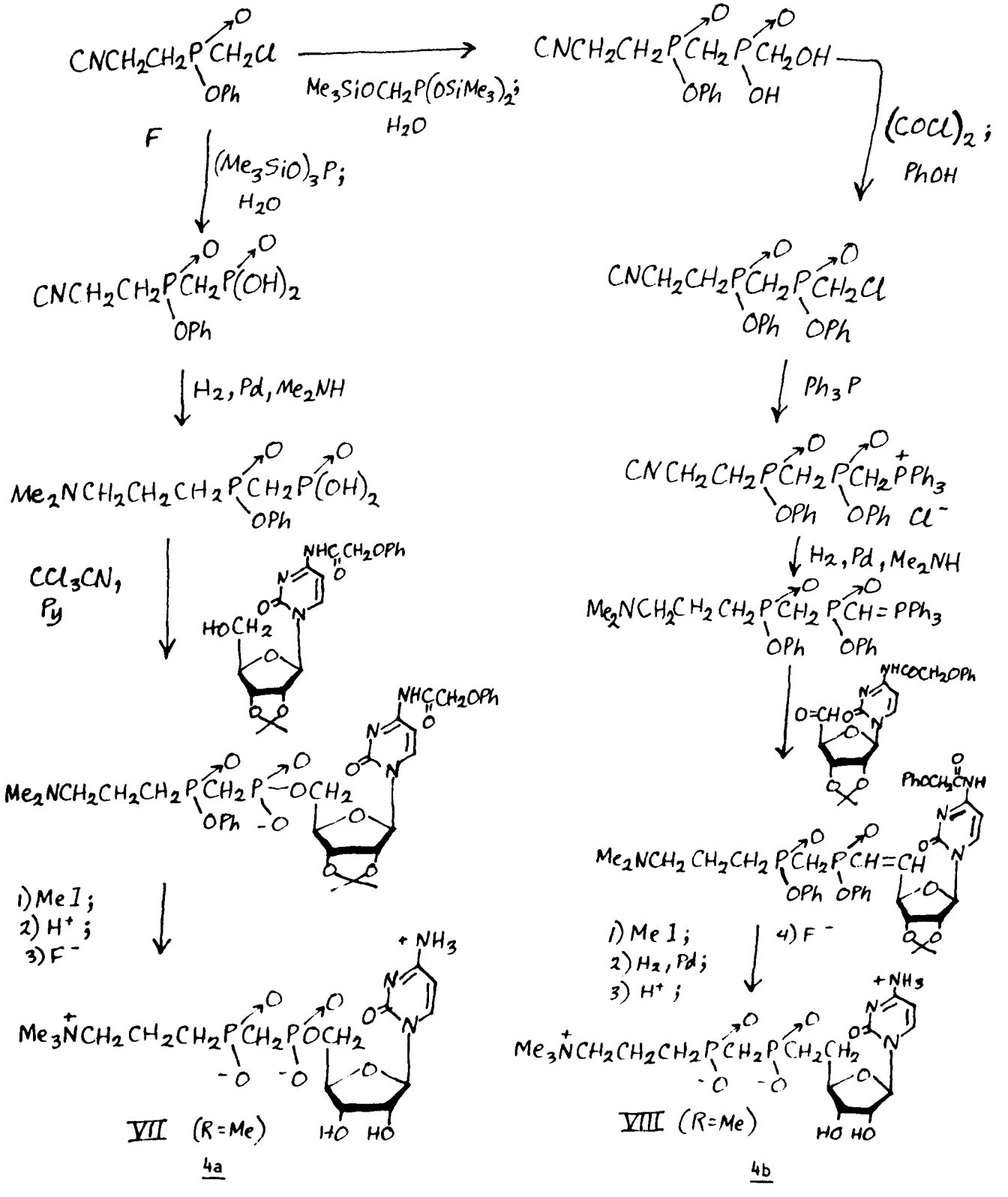

CNCH2CH2P(O)(OPh)CH2Cl
Me3SiOCH2P(OSiMe3)2; H2O
F
(Me3SiO)3P; H2O
CNCH2CH2P(O)(OPh)CH2P(O)(OH)2
H2, Pd, Me2NH
Me2NCH2CH2CH2P(O)(OPh)CH2P(O)(OH)2
CCl3CN, Py
NHC(O)CH2OPh
HOCH2
Me2NCH2CH2CH2P(O)(OPh)CH2P(O)(-O)OCH2
1) MeI; 2) H+; 3) F-
+NH3
Me3+NCH2CH2CH2P(O)(-O)CH2P(O)(-O)OCH2
VII (R=Me)
HO HO
4a
CNCH2CH2P(O)(OPh)CH2P(O)(OH)CH2OH
(COCl)2; PhOH
CNCH2CH2P(O)(OPh)CH2P(O)(OPh)CH2Cl
Ph3P
CNCH2CH2P(O)(OPh)CH2P(O)(OPh)CH2+PPh3 Cl-
Me2NCH2CH2CH2P(O)(OPh)CH2P(O)(OPh)CH=PPh3
O=CH
NHCOCH2OPh
PhOCH2CONH
Me2NCH2CH2CH2P(O)(OPh)CH2P(O)(OPh)CH=CH
1) MeI; 2) H2, Pd; 3) H+; 4) F-
Me3+NCH2CH2CH2P(O)(-O)CH2P(O)(-O)CH2CH2
VIII (R=Me)
HO HO
4b

Fig. 4

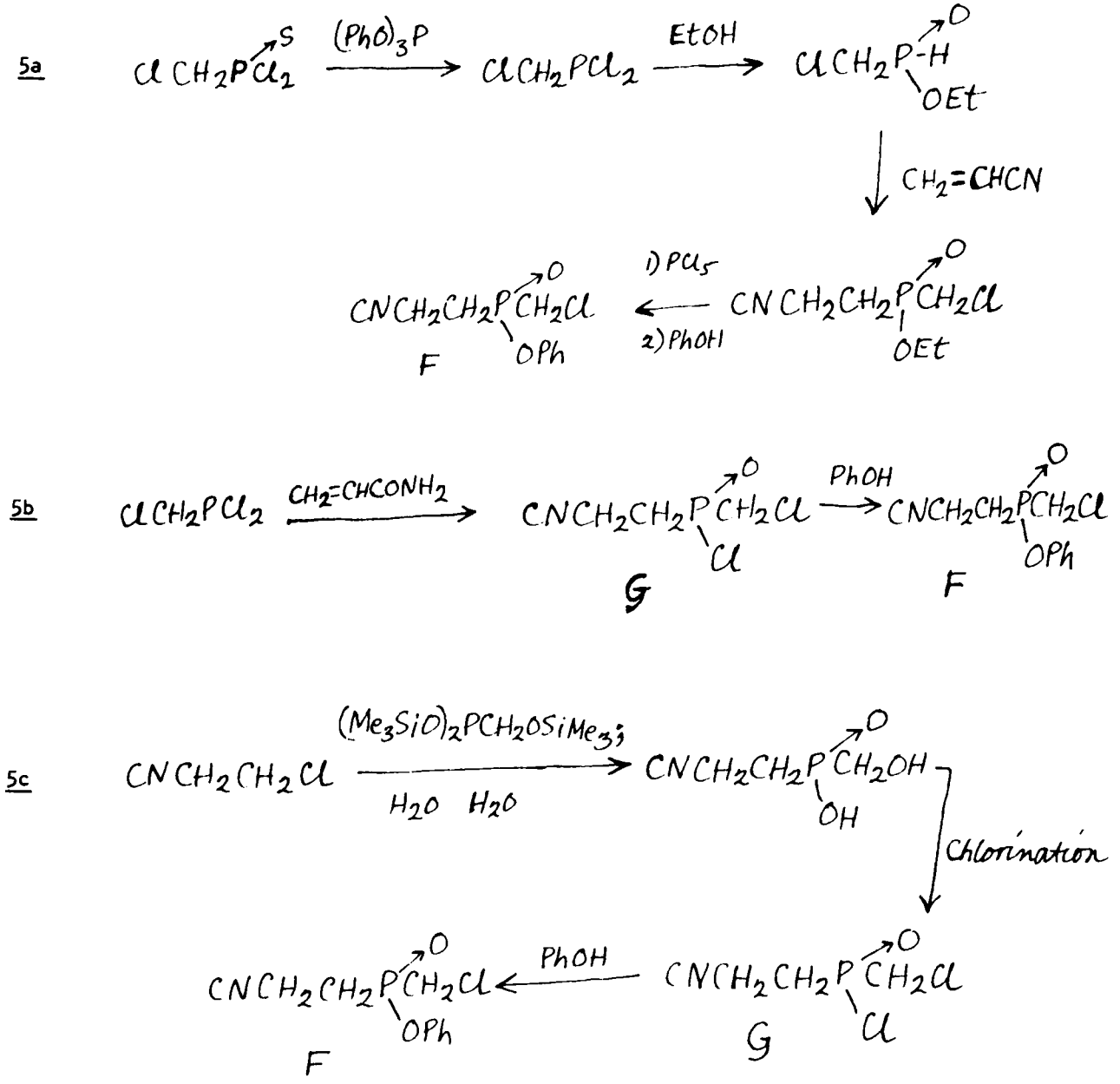

5a
ClCH2PCl2
(PhO)3P
ClCH2PCl2
EtOH
ClCH2P(O)(H)(OEt)
CH2=CHCN
CNCH2CH2P(O)(CH2Cl)(OEt)
1) PCl5
2) PhOH
CNCH2CH2P(O)(CH2Cl)(OPh)
F
5b
ClCH2PCl2
CH2=CHCONH2
CNCH2CH2P(O)(CH2Cl)(Cl)
G
PhOH
CNCH2CH2P(O)(CH2Cl)(OPh)
F
5c
CNCH2CH2Cl
(Me3SiO)2PCH2OSiMe3;
H2O H2O
CNCH2CH2P(O)(CH2OH)(OH)
Chlorination
CNCH2CH2P(O)(CH2Cl)(Cl)
G
PhOH
CNCH2CH2P(O)(CH2Cl)(OPh)
F

Fig. 5

illustrates the route which a priori appeared to be capable of giving the desired product with the least effort. Chloromethylphosphonous dichloride was easily prepared by published procedure, which involved thiation of chloromethylphosphonic dichloride with phosphorus pentasulfide, followed by desulfurization with triphenyl phosphite. (Chloromethylphosphonic dichloride, however, is no longer a commercial product, since its manufacture involves the probable byproduct formation of bis(chloromethyl)ether, a potent carcinogen; this problem is detailed in the third progress report).

Chloromethylphosphonous dichloride was readily converted to monoethyl hydrogen chloromethylphosphonite by treatment with ethanol in the absence of base. This compound, which has not previously been reported, had a bp at 48-50° C at .05 torr. IR and nmr agreed with the structure; the methyl ester is a known compound. Attempts to produce addition of this monoester to acrylonitrile (itself, incidentally, a suspect substance of late) by a variety of base-catalytic conditions failed to yield any detectable expected addition product. Conditions employed included catalytic amounts of aqueous sodium hydroxide; sodium hydroxide-tetrabutylammonium bisulfate; and the non-nucleophilic diisopropylethylamine, among others. In some cases considerable heat was evolved on addition of the catalyst, and some new reaction products appeared; however, on gas chromatographic analysis, none of these could reasonably be ascribed to the desired addition product, and it was concluded that polymerization of acrylonitrile was the predominant reaction observed.

The reported reaction of chloromethylphosphonous dichloride (5) with acrylamide to give the dichloride G was next tried. The reaction mixture became very viscous, black, and completely failed to yield any significant amount of the desired product on attempted high vacuum distillation. It was thereby concluded, by a process of elimination, that route 5c would have to serve

to produce the important intermediate. A satisfactory reaction of 3-chloropropionitrile with bis(trimethylsily)trimethylsilyoxymethylphosphonite had, of course, previously been observed, yielding on aqueous hydrolysis cyanoethyl(hydroxymethyl)phosphinic acid (9). Careful subsequent investigation showed that the acid is usually contaminated with byproducts which may be difficult to remove at a later stage and which may even interfere with subsequent reactions, however. Thus, it became important to purify the acid as much as possible before further synthetic steps were undertaken.

Purification was accomplished by recrystallization several times from acetone-hexane mixtures from room temperature to 5^{o}. The procedure was accompanied by appreciable losses of material, so that it was not entirely optimal,but was the most satisfactory found. Fortunately, purity of the acid could be checked quite easily by gas chromatography after silylation with bis(trimethylsily)trifluoroacetamide. None of the samples of the acid were grossly contaminated, and all could be purified to a satisfactory state of homogeneity by recrystallization. The product appeared at a retention time of 4.9 minutes (3% OV-17, 170^{o}) while all the impurities appeared at shorter retention times. Evidence cited below indicates strongly that the two major and most pertinent impurities were 2-cyanoethylphosphonic acid and hydroxymethylphosphonic acid; the latter almost certainly arises from oxidation and hydrolysis of the starting silylreagent.

Conditions under which cyanoethyl(hydroxymethyl)phosphinic acid could be chlorinated and converted to its phenylester to produce the intermediate F were the subject of a great deal of investigation as well. Treatment with thionyl chloride followed by phenol in pyridine completely failed to give the desired material in any appreciable yield. Milder conditions using triphenylphosphine and carbon tetrachloride,followed by phenol, gave a complex mixture which also concluded not to be preparatively useful. It seems to be clear

after considerable study that the presence of a base, at least for the phenylation,was undesirable. Besides pyridine and triethylamine, the non-nucleophilic bases diisopropylethylamine and 2,6 di-tert-butylpyridine were tried, all without success.

Finally, a very simple procedure was found to give the product quite satisfactorily, by the use of oxalyl chloride for chlorination; and following removal of excess reagents in vacuo, phenol was added and the mixture warmed for some hours under reduced pressure to remove the liberated hydrogen chloride. Excess phenol was removed from the reaction product after cooling by repeated extraction with sodium bicarbonate solution, followed by high vacuum sublimation of any residual phenol.

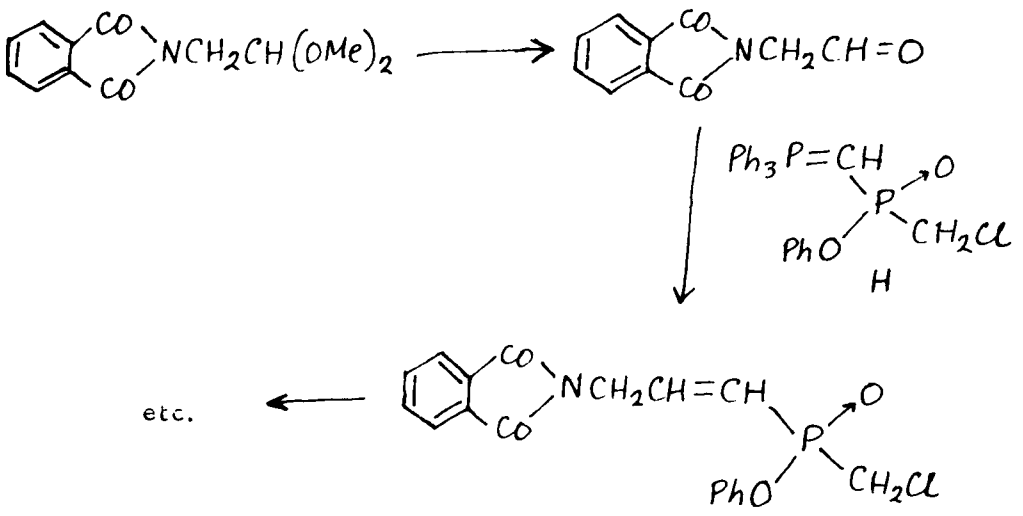
The composition of the crude reaction product could be followed readily by gas chromatography, using both a flame ionization detector and a phosphorus-sensitive detector. On 3% OV-17 at 200°, the product eluted at 12.0 min. In some samples, one or two contaminants were also noted; the earlier one eluting at 13.5 min. and the later one eluting at this temperature at 33.1 min. Combined gas chromatography-mass spectroscopy confirmed the main peak as the desired product and showed that the 13.5 min. peak was diphenyl chloromethyl-phosphonate,which apparently arises from an excess of the original silyl reagent remaining in the reaction mixture prior to hydrolysis.

The 33.1 min. impurity was more interesting and was found to be diphenyl cyanoethylphosphonate. This substance almost certainly arose at the stage of Arbuzov reaction; it must have arisen by contamination of the silyl reagent with tris(trimethylsilyl)phosphite. Except for the fact that the silyl reagent was twice distilled before use, and tris(trimethylsilyl)phosphite has a distinctly lower boiling point, the presence of the latter is most logically explained by contamination of the hypophosphorous acid at the first step

with phosphorous acid. Possibly on storage, some sort of disproportionation leading to tris(trimethylsilyl)phosphite formation may have occurred as well.

Methods to completely purify the main product fraction had just begun to be studied at the conclusion of the first year's work. In any event, the impurities were largely minimized by the recrystallization procedure described above for the phosphinic acid.

A secondary access route into the cytidine diphosphate choline analog was investigated for a time during the fourth quarter. This begins with the commercially available phthalimidoacetaldehyde dimethylacetal:



The acetal was satisfactorily hydrolyzed to the corresponding aldehyde, but reaction with the ylid H gave a number of products in addition to what was apparently the desired compound. Triphenylphosphine oxide (revealed by gas chromatography) contaminated the reaction mixture and, like the other contaminants,

was very difficult to remove. No satisfactory purification procedure could be found; and since in addition the reaction did not seem to go in satisfactory yield, this route was at least temporarily set aside.

EXPERIMENTAL

Only the portion of the annual report which deals with the fourth quarter's work is discussed in this section. The preceding three quarterly reports give full experimental details for the work done during those respective periods.

1,2-Dihexadecyl-sn-glycerol-3-phosphoryl-1'-methylcholine, IV.

1,2-Dihexadecyl-sn-glycerol. 1,2-Dihexadecyl-sn-glycerol was prepared from D-mannitol by the method of Chen and Barton(6).

Diphenyl 1,2-Dihexadecyl-sn-glycerol-3-phosphate. To a solution of 1,2-dihexadecyl-sn-gylcerol (1080 mg; 2.mmols) in 25 ml pyridine-chloroform (4:1; v/v) was added diphenylchlorophosphate (540 mg; 2. mmols). The solution was stirred at room temperature for 24 hr. The solvents were removed at reduced pressure.

1,2-Dihexadecyl-sn-glycerol-3-phosphate. Diphenyl 1,2-dihexadecyl-sn-glycerol-3-phosphate (1540 mg; 2. mmols) was dissolved in 100 ml warm glacial acetic acid. This solution was slowly added to a vigorously stirred suspension of reduced platinum in glacial acetic acid (100 ml) and reduced with hydrogen at room temperature and atmospheric pressure. When hydrogen was no loger taken up, chloroform (200 ml) was added and the mixture filtered through a sintered glass filter. The platinum was washed with another 25 ml of chloroform. The filtrate and washings were combined and the solvents removed at reduced pressure. The remaining white solid was dissolved in 2-propanol and again taken to dryness at reduced pressure.

1,2-Dihexadecyl-sn-glycerol-3-phosphoryl-1'-methylcholine. To a solution of 1,2-dihexadecyl-sn-glycerol-3-phosphate (1240 mg; 2 mmols) in pyridine (50 ml) was added a 10-fold excess (5.8 g) of 2-hydroxy-1-(trimethyl amino) propane tosylate salt (7). Trichloroacetonitrile (20 ml) was added and the suspension was stirred at 50° C for 48 hr, during which time the mixture turned dark brown. Removal of the solvents at reduced pressure yielded a dark brown viscous oil. Acetonitrile (100 ml) was added and the mixture allowed to stand at 4° overnight. The tan colored precipitate was removed by filtration on a sintered glass filter and dried in vacuo overnight. The waxy solid was dissolved in tetrahydrofuran-water (9:1, v/v) and applied to a column (1.5 cm x 40 cm) of Amberlite MB-3 resin previously washed with the same solvent. The column was eluted with 500 ml THF-water (9:1, v/v) at a flow rate of 2 ml/min. The colorless eluate was concentrated under reduced pressure and dried by repeated azeotrophic distillation with 2-propanol at reduced pressure. The waxy white solid was dissolved in chloroform and applied to a column of silic AR CC-7. The column was first eluted with 500 ml chloroform-methanol (4:1, v/v) to remove unreacted 1,2-dihexadecyl-sn-glycerol-3-phosphate. Elution of the column with chloroform-methanol (2:3, v/v) removed the desired product. After removal of the solvent, the residue was redissolved in chloroform and passed through a Metricel^{TM} Alpha-6 (.45μm) filter to remove suspended silicic acid. The solvent was removed, leaving a chromatographically pure (TLC on silica gel G, chloroform-methanol-water, 65:25:4, v/v/v) white solid. Yield 1123 mg (78% from 1,2-dihexadecyl-sn-glycerol). M.p. 206-208° C (dec.).

Anal. Calcd. for $C_{41}H_{86}O_6NP \cdot H_2O$ (738.129):

C, 66.72; H, 12.15; N, 1.90;, P, 4.20

Found:

C, 66,35; H, 11.95; N, 1.98; P, 4.20.

Condensation of L-3,4-dioctadecoxybutyl(phenoxyphosphino)methylphosphonic acid with N-phenoxyacetyl-2',3'-isopropylidenecytidine. The purified phosphonic acid B (35 mg, 0.042 mmol) and 4 N-phenoxyacetyl-2',3'-isopropylidenecytidine (51 mg, 0.122 mmol; 3 X excess) were dissolved in 40 ml of anhydrous pyridine and to this solution was added trichloroacetonitrile (1.5 ml). The solution was stirred at 53° overnight.

The solvent was removed in vacuo and the residue precipitated with acetonitrile. Little or no condensation product was evident on TLC in chloroform-methanol-water-acetic acid, 80:13:8:0.3. The precipitate (28 mg) consisted of the starting phosphonic acid as a salt. The acetonitrile solution contained the cytidine derivative,which was recovered as well.

The acid salt (28 mg, 3.4×10^{-5} mol) was dissolved with a fresh portion of the cytidine derivative (52 mg, 12.6×10^{-5} mol) and to the mixture was added 2 ml of trichloroacetonitrile. This time the reaction was carried out overnight at 70°. TLC of the residue remaining after removal of volatile materials showed the condensed product with an Rf similar to that of the model compound (see text above). The product was precipitated again by addition of acetonitrile; the cytidine derivative was again recovered from the solution. The precipitated product (11 mg) gave essentially one spot which was phosphorus-positive and UV-absorbing at Rf 0.75 in the solvent mixture employed above.

Hydrolysis of the Condensation Product E. The crude condensation product obtained by acetonitrile precipitation (10 mg) was dissolved in 1 ml of trifluoroacetic acid containing 2 drops of water and the mixture heated at 45° for 24 hrs. Evaportion of the acid, followed by dehydration twice with isopropyl alcohol gave a residue which was dissolved in a minimum amount of chloroform. Addition of ether gave a solid which was filtered off and dried in vacuo after thorough washing with cold ether. This material gave a single spot which was UV- and phosphorus-positive by TLC in the above solvent mixture; Rf 0.6.

This value is similar to that of authentic phosphonic acid analogs of CPD-diglyceride (8) and chraacterization of the product was underway as the quarter ended.

Reaction between L-3,4-dioctadecoxybut-3-enyl(chloromethyl)phosphinic acid phenyl ester and bis(trimethylsily)trimethylsilyoxymethylphosphonite. The crude chloromethyl compound (2.3 g, 2.9 mmol) was reacted with 10 ml (10X excess) of the freshly distilled silyl reagent under a nitrogen atmosphere at 130°. After 1 hr, a few drops of trimethylsilyl chloride had distilled. The temperature of the bath was increased to 135° and the reaction mixture left overnight. After cooling, the excess reagent was removed by distillation in high vacuum, and the insoluble residue dissolved in tetrahydrofuran (9 ml). To the solution was added water (5 ml) and the clear solution left overnight. The solvent was evaporated and water was removed by repeated re-evaportion with isopropyl alcohol. The residual white solid was dissolved in a minimum quantity of chloroform and the product precipitated with acetonitrile. Approximately 1.7 g of material was obtained in this way; its thin-layer chromatographic behavior was similar to that of 1-octadecoxy-2-hexadecoxypropyl phosphonic acid. Final characterization of this important intermediate had not yet been completed at the end of the fourth quarter.

The crude product was hydrogenated in a warm (50°) mixture of tetrahydrofuran-acetic acid (1:1) with 0.5 g of 10% paladium on charcoal at 50 lb hydrogen pressure overnight.

After filtration and washing of the catalyst, the solvents were thoroughly evaporated and the residue reprecipitated with acetonitrile. This experiment was carried out just at the very end of the fourth quarter, and therefore further steps in the characterization of the hydrogenated product extended beyond the period covered by this report.

Chloromethyl ethyl hydrogen chloromethylphosphonite.

Chloromethyl phosphonous dichloride was obtained in about 60% yield starting with the procedure published in Organic Syntheses (10). To 8 ml of the dichloride cooled in an ice bath was added, drop wise under a nitrogen atmosphere, 50 ml of absolute ethanol freshly distilled from calcium hydride. After stirring for a few minutes further, excess ethanol was distilled off in vacuo at water pump pressure and the product distilled in high vacuum; $b_{0.05}$ 48-50°. Infrared and nmr data were consistent with the supposed structure, although gas chromatography indicated that the product was impure.

This crude material, on attempted reaction with acrylonitrile in the presence of diisopropylethylamine, or of sodium hydroxide in catalytic amount with or without tetrabutylammonium bisulfate, failed to give any product with the expected properties although some type of reaction had obviously occurred in the latter cases.

An attempt to prepare chloromethyl cyanoethyl phosphinic chloride by heating chloromethylphosphonous dichloride with acrylamide according to the procedure of Pudovik et al (5) failed to give the desired product from the black tarry reaction mixture.

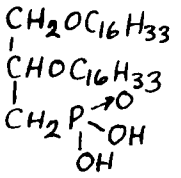
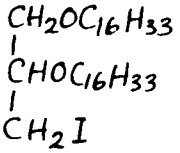
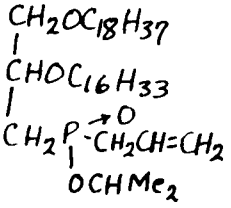
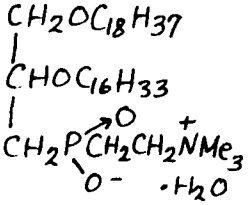
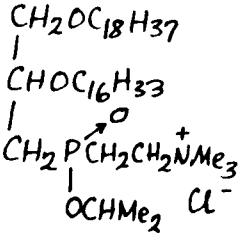
Thus, the desired phenyl cyanoethyl(chloromethyl)phosphonate was prepared according to the procedure given in the third quarterly report, starting from cyanoethyl(hydroxymethyl)phosphinic acid synthesized as reported in the same place. The acid failed to form a crystalline cyclohexylamine salt, so that purification of the free acid had to be accomplished by repeated crystallization from acetone-hexane.

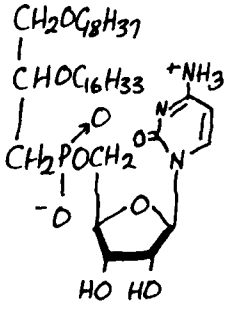
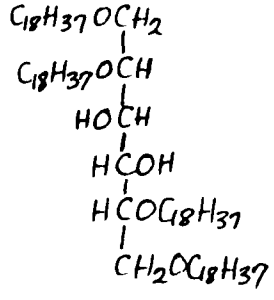
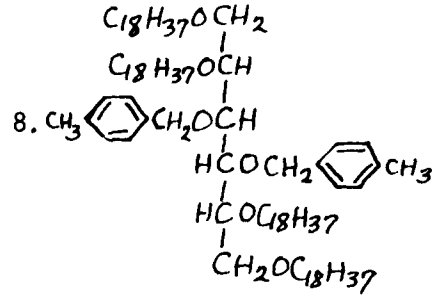
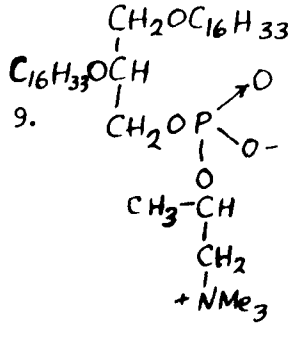
Attempted reaction between phthalimidoacetaldehyde and phenyl chloromethyl-(triphenylphosphinemethylide)phosphinate.

The aldehyde was prepared from its dimethylacetal by hydrolysis with hydrochloric acid in dioxane solution. The ylid was prepared correspondingly from its phosphonium

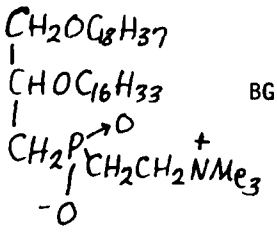
chloride. Reaction between the aldehyde and ylid at 110° for 24 hrs gave a mixture of products, of which none was predominent. Triphenylphosphine oxide was clearly visible by TLC and gas chromatography. Various chromatographic separation procedures were tried without any clear success in isolating the desired product.

COMPOUNDS SUBMITTED FOR ANTIMALARIAL TESTING

	COMPOUND	BOTTLE NO.	DATE TESTED	TEST RESULTS
1.		BG 37644	6-4-76	Inactive. Non-toxic.
2.		BG 79188	12-16-76	Inactive. Non-toxic.
3.		BG 84317	2-23-77	Inactive. Non-toxic.
4.		BG 82182	2-10-77	Inactive. Non-toxic.
5.		BG 81777	2-17-77	Inactive. Non-toxic.

COMPOUND	BOTTLE NO.	DATE TESTED	TEST RESULTS
6. 	BH 08611	6-7-77	Inactive. Non-Toxic.
7. 		Submitted 1-77. Not tested yet.	
8. 		Submitted 5-77. Not tested yet.	
9. 	BH 16597	Submitted 6-1-77 Not tested yet.	

COMPOUND SUBMITTED FOR LEISHMANIASIS TESTING

	COMPOUND	BOTTLE NO.	DATE TESTED	TEST RESULTS
1.		BG 83182	3-11-77	48.8% suppression at 52 mg/kg; 93% suppression at 208 mg/kg Not considered sufficiently active. No toxicity.

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FIFTH QUARTERLY REPORT: DAMD-17-76-C-6073

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PERIOD: July 1, 1977 - September 30, 1977

During the fifth project period, our efforts were concentrated along three fronts: (a) preparation of intermediates for synthesis of the phosphinate phosphonate analogs of CDP-ethanolamine and CDP-choline (see scheme 1); (b) preparation of lipid intermediates for the synthesis of isosteric analogs of CDP-diglycerides (see Scheme 2); and (c) studies on the oxidation of appropriately blocked derivatives of cytidine for use in the synthesis of phosphinate phosphinate analogs of CDP-diglycerides (see Scheme 3).

The availability of 3.5 kilograms of trimethylsilyldiethylamine has allowed facile production of a relatively large amount of bis(trimethylsilyl)trimethylsilyloxymethylphosphonite needed for the synthesis of the first and second partsof this report.

Large scale reaction of bis(trimethylsily)trimethylsilyloxymethyl phosphonite with 3-chloropropionitrile (scheme 1) and subsequent hydrolysis of the product has allowed the preparation of relatively large amounts of 2-cyanoethyl(hydroxymethyl)phosphinic acid. A modified chlorination and phenylation procedure has yielded virtually pure 2-cyanoethyl(chloromethyl)phenyl phosphinate insofar as no other phosphorus-containing compounds were formed in the reaction.

The second part of the work concerns the preparation of isosteric analogs of CDP-diglycerides (see Scheme 2).

From the hydrogenation of crude XIV (see annual Progress Report pg 22) we obtained product XV, which after recrystallization from hot hexane was sent for elemental analysis. Since the phosphorus analysis was high (97% found vs. 7.35% calculated), a treatment with trifluoroacetic acid was carried out to break any possible pyro compounds that might be present. Thin layer chromatography of the acid treated product revealed it to be identical to the

starting material. Purification of XV by column chromatography led to the isolation of a chromatographically homogeneous (TLC) material that gave a very good elemental analysis for the required compound; however, nuclear magnetic resonance spectroscopy of a tetrachloroethene solution of the compound showed no phenyl absorption.

It appears that the loss of the phosphinic phenyl ester group occurred either in the Arbuzov reaction or in the hydrogenation step. In order to test this hypothesis, a new small scale preparation of XIV from the crude chloromethyl and freshly prepared silyl reagent (see p. of this report) was undertaken. Elemental analysis of the column purified product XIV gave the correct values for carbon and hydrogen but not for phosphorus (we are about to do our own phosphorus analysis to check this result).

The surprising fact is that NMR spectroscopy of XIV shows no phenyl absorption. Every step of this sequence of steps is being reviewed to locate the step where the phenyl group is lost.

Since reaction of XV with oxalyl chloride followed by phenol should yield XVI even without the protection of the phenyl group, we are proceeding to try this reaction. We are currently in the process of finding the experimental conditions for this reaction and in the determination of the products.

The last part of this report discusses work that has been done on the synthesis of the phosphinate phosphinate analogs of CDP-diglycerides (see Scheme II, original research proposal). During the fifth project period, we did not have the N4-phenoxyacetyl-2',3'-isopropylidene cytidine that is the desired intermediate for this synthetic route. However, in order to optimize the reaction conditions for the oxidation of the C-5 primary alcohol on the ribose moiety appropriately blocked cytidine derivative, several studies were carried out on the oxidation of N4-acetyl-2',3'-isopropylidene cytidine which

would be expected to react in a similar fashion to the N4-phenoxyacetyl derivative. These studies centered around the oxidation of N4-acetyl-2',3'-isopropylidene cytidine to N4-acetyl-2',3'-isopropylidene cytidine-5'-carboxaldehyde by use of dicyclohexylcarbodiimide and dimethyl sulfoxide as described by Jones and Moffatt. Reaction of N4-acetyl-2',3'-isopropylidene cytidine-5'-carboxaldehyde with dianilinoethane formed the crystalline diphenylimidazolidine derivative. This derivative was isolated from the reaction mixture in a partially pure form.

The availability of 80 gm of N4-phenoxyacetyl-2',3'-isopropylidene cytidine has allowed us to carry on with these studies using this more suitable synthetic intermediate in later work.

EXPERIMENTAL

Preparation of Hydroxymethylphosphonous Acid. To 128 ml of commercial 50-52% hypophosphorous acid (found to contain 65 g H_3PO_2 per 100 ml) (1.26 mol H_3PO_2) was added portionwise over a period of two hours a total of 38 g (1.26 mol) of paraformaldehyde. During the addition (under static N_2 pressure) and magnetic stirring, the temperature of the reaction mixture was maintained at 40-45°C. After all the paraformaldehyde had been added, the reaction mixture was stirred at 40-45° overnight. Water was removed under reduced pressure by repeated azeotropic distillation with 2-propanol. Yield of mixed products, 110 g.

Preparation of Bis(trimethylsilyl)trimethylsilyloxymethylphosphonite. The crude hydroxymethylphosphonous acid (110 g; 1.15 moles) was transferred to a 2 liter 3-necked flask with 80 ml acetonitrile (distilled from P_2O_5). The flask was mounted over a magnetic stirrer and water bath for cooling.

The flask was fitted with a reflux condenser, a 500 ml dropping funnel, and a nitrogen inlet (N_2 outlet through drying tube mounted on top of reflux condenser). N,N-Diethylaminotrimethylsilane (711 g; 4.9 moles; 1.42 times required amount) was added dropwise over a period of four hours. Very little heat was generated during addition. The mixture was allowed to stir overnight at room temperature, then refluxed with stirring for two hours and allowed to cool to room temperature. The apparatus was fitted for vacuum distillation with a long vigreux distillation head and condenser. Application of water pump vacuum removed diethylamine as a gas. Excess trimethylsilyldiethylamine distilled at 40-45°. Distillation apparatus was connected to a mechanical pump through traps. Bis(trimethylsilyl)-trimethylsilyloxymethylphosphonite distilled between 60 and 80° C.
The crude bis(trimethylsilyl)trimethylsilyloxymethyl phosphonite was redistilled using a Nester-Faust spinning band column. Fractions collected: (1) b.p. 0.030 30-40°; ~10 ml; (2) b.p. 0.050 40-43; ~15 ml; (3) b.p. 0.020 43-44; ~250 ml; (4) approx. 10-15 ml remained in distillation flask. Fraction (3) was taken as the product. Yield of bis(trimethylsilyl)trimethylsiloxymethylphosphonite 208 g (58%), by far the highest yield of this intermediate we have ever obtained.

Preparation of 2-Cyanoethyl(chloromethyl)phenylphosphinate (VI).
2-cyanoethyl(hydroxymethyl)phosphinic acid was prepared by a modification of the chlorination and phenylation procedure described in the third quarterly report. Recrystallized 2-cyanoethyl(hydroxymethyl)phosphinic acid (5.14 g; 0.034 mol) was dissolved with warming in 100 ml dry acetonitrile (freshly distilled from P_2O_5). A small amount of the phosphinic acid remained in suspension. Oxalyl chloride (60 ml; 0.71 mol) was added dropwise over a period of 20 min with rapid magnetic stirring at room temperature. During the addition, a waxy white solid precipitated. After all the oxalyl chloride had been added, a drop of N,N-dimethylformamide and a drop of 2,6-di-t-butylpyridine were added and

the mixture was refluxed (70° oil bath) for one hour, during which time all of the previously precipitated white solid dissolved. The clear, slightly brown solution was allowed to cool to room temperature. Acetonitrile and excess oxalylchloride were removed on a rotary evaporator (bath temp, approximately 40° C).

Phenol (5.0 g; 0.053 mol) was added and the darkening mixture heated in a 60° C oil bath (under water aspirator vacuum to remove HCl gas) until the evolution of HCl had stopped (about two hours). The mixture was cooled to room temperature and 250 ml of hydrocarbon-stabilized chloroform was added to the viscous dark residue. The mixture was shaken vigorously for about 15 minutes and the undissolved solid removed by filtration through Whatman No. 54 paper. The dark brown chloroform filtrate was extracted twice with 100 ml volumes of saturated aqueous sodium bicarbonate followed by two extractions with 50 ml of saturated aqueous sodium sulfate. The organic phase was dried with anhydrous $MgSO_4$ and filtered through Whatman No. 52 paper. Removal of the chloroform under reduced pressure gave a dark brown oil (4.31 g). A small amount of the oil was dissolved in ethyl acetate and subjected to gas-liquid chromatography on a 6 ft. column of OV-17 at 200° C. This showed that the preparation was virtually pure in that no other phosphorus-containing compounds were present, but it did contain unreacted phenol and some diphenyl oxalate. For this reason, we are currently working on a chromatographic purification of the chloromethyl compound.

Compound XV (1 g; from the hydrogenation of crude XIV: see Annual Progress Report p.22) was recrystallized from 25 ml hot hexane. Thin layer chromatography indicated that the low polarity, nonphosphorous containing impurity had been removed. Elemental analysis gave 9.75 P vs. the 7.35% expected.

Purification of XV: Crude XV (0.230 g recrystallized from hot hexane) was applied to a 25 g column (2 X 25 cm) of SilicAR CC-7 and eluted with chloroform; chloroform-methanol, 90:10; chloroform-methanol, 85:15; chloroform-methanol, 80:20. Fractions 22-27 contained a chromatographically pure phosphorus-containing compound (Rf 0.5, TLC in chloroform-methanol-formic acid, 44.5:5:0.5).

Anal. Calcd. for $C_{48}H_{92}O_7P_2$: C, 68.37; H, 10.99; P, 7.34. Found: C, 68.54; H, 11.58; P, 6.5 ± 0.6%. Nuclear magnetic resonance spectrum of the sample in tetrachloroethene does not show phenyl absorption.

New Reaction between L-3,4-dioctadecoxybut-3-enyl(chloromethyl)phosphinic acid phenyl ester and bis(trimethylsilyl)trimethylsilyloxymethylphosphonite. Procedure and reaction conditions were identical to those described in Annual Progress Report, p 22. Amounts: chloromethyl compound: 0.460 g; 0.51mmol; bis(trimethylsilyl)trimethylsilyloxymethylphosphonite: 2 ml (10 fold excess). 0.420 g of crude XIV was precipitated with cold acetonitrile. Thin layer chromatography showed the expected compound plus a low-polarity non phosphorus-containing impurity and a polar phosphorus containing impurity that streaks from the origin. No chloromethyl starting material remained in the reaction mixture.

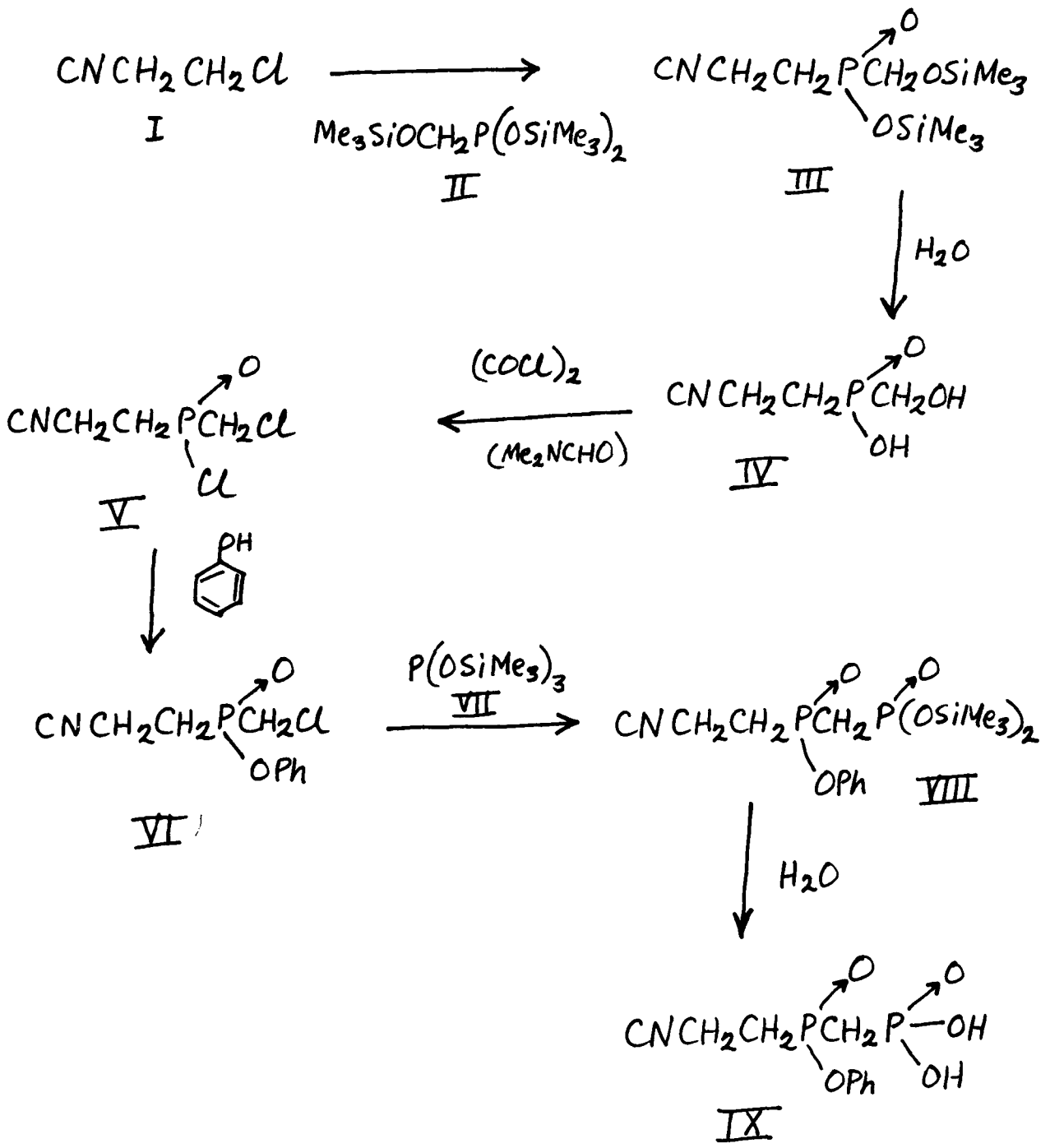
Purificat'on of XIV. Crude XIV (0.42 g in 6 ml chloroform) was applied to a 45 g column (2 X 35 cm) of SilicAR CC-7. The column was eluted with chloroform; chloroform-methanol, 95:5; chloroform-methanol, 90:10; chloroform-methanol, 85:15. Fractions 22-25 contained a chromatographically pure, phosphorus-containing compound.

Anal calc'd for $C_{48}H_{90}O_7P_2$: C, 68.54; H, 10.78; P, 7.36. Found: C, 68.05; H, 10.79; P, 3.99. NMR in tetrachloroethene shows no phenyl absorption. Our own phosphorus analysis is being done on these compounds.

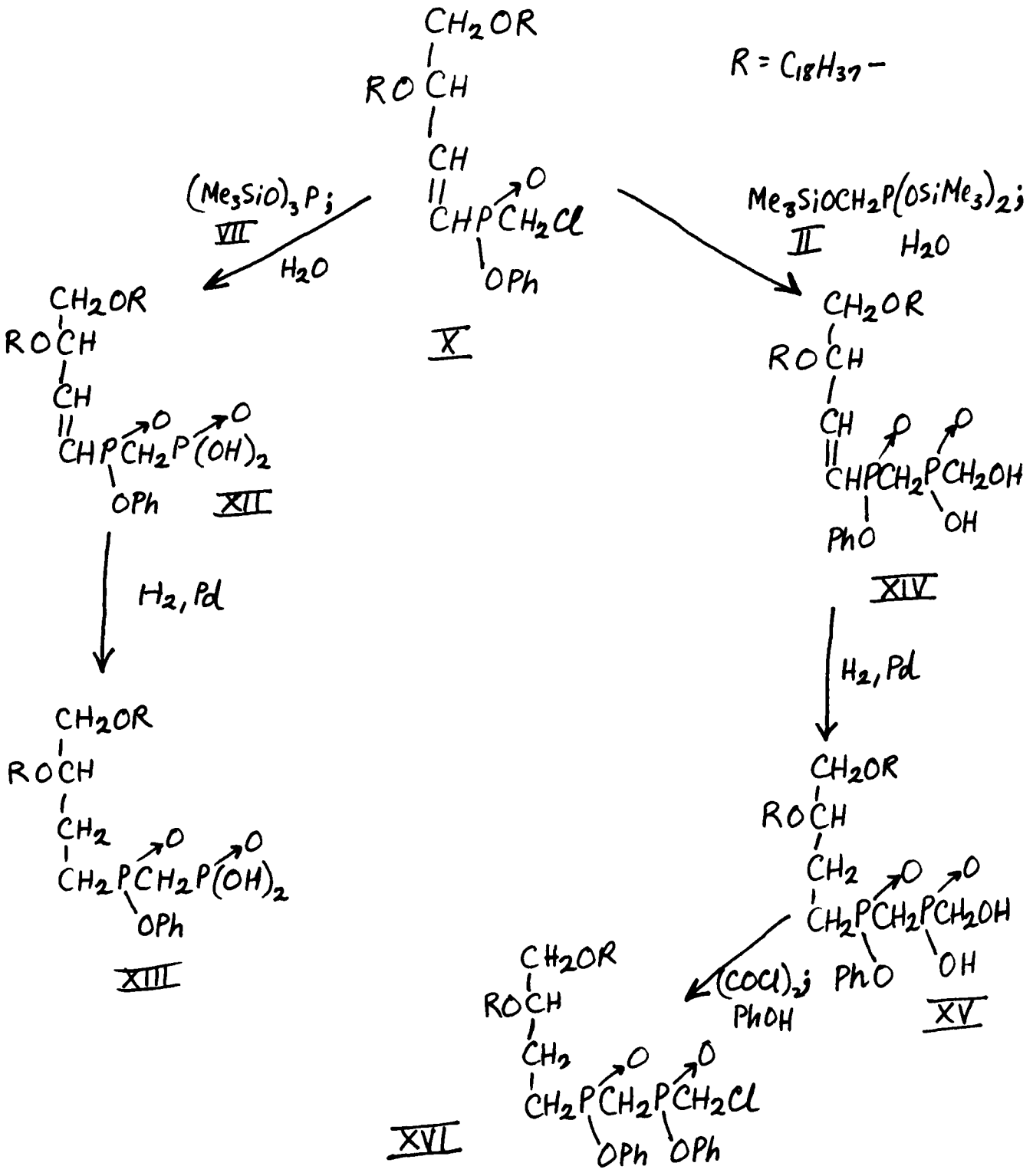
Oxidation of N4-Acetyl-2',3'-isopropylidene cytidine. N4-Acetyl-2',3'-isopropylidene cytidine (163 mg; 0.50 mmol) and dicyclohexylcarbodiimide (309 mg; 1.5 mmol) were dissolved in 1.2 ml DMSO (dried) and 1.0 ml methylene chloride (also dried over molecular sieve) under a nitrogen atmosphere with vigorous magnetic stirring. The solution was immersed in an ice-water bath. No precipitate was observed. To the solution at 5° was added 20 µl of dichloroacetic acid. Almost at once, a precipitate began to form. After 5 min, the reaction mixture was allowed to warm to room temperature and stirred for another 1.5 P.

While stirring vigorously, a solution of oxalic acid (126 mg; 1 mmol) in 1.5 ml methanol was added. The mixture was stirred at 21° for 30 minutes. The mixture was then filtered and the precipitate washed with a little cold methanol. A solution suspension of 127 mg of dianilinoethane (recrystallized) in 2 ml methanol was added to the filtrate. The mixture was partially evaporated to remove methylene chloride and the residue, consisting mainly of DMSO, was diluted with 3 ml methanol. Even after standing overnight at -5°, only a small crystalline precipitate was observed. This had first appeared at room temperature upon addition of water. After some days at -5°, more crystals appeared. The precipitate was filtered off. Additional material (designated "methanol soluble") was obtained by addition of water and cooling. NMR spectra suggested the initial material to be impure product.

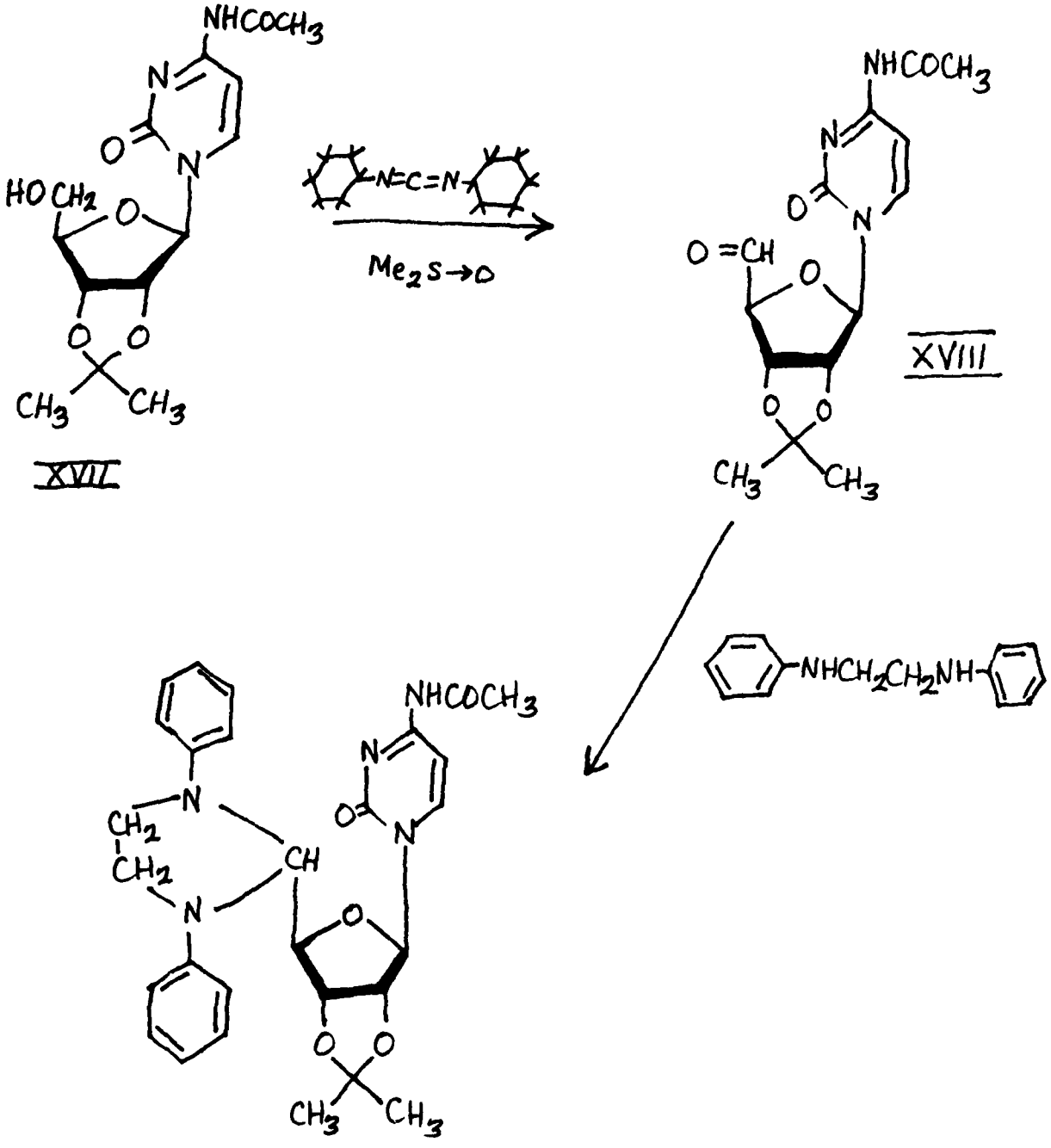
Since it was recognized that N4-phenoxyacetyl and not the N4-acetyl derivative would be the probable synthetic intermediate, no exhaustive effort was made to purify the N4-acetyl product but to await the availability of the N4-phenoxyacetyl-2',3'-isopropylidene cytidene derivative.


CNCH2CH2Cl
I
Me3SiOCH2P(OSiMe3)2
II
CNCH2CH2P(O)CH2OSiMe3
OSiMe3
III
H2O
CNCH2CH2P(O)CH2OH
OH
IV
(COCl)2
(Me2NCHO)
CNCH2CH2P(O)CH2Cl
Cl
V
PH
CNCH2CH2P(O)CH2Cl
OPh
VI
P(OSiMe3)3
VII
CNCH2CH2P(O)CH2P(O)(OSiMe3)2
OPh
VIII
H2O
CNCH2CH2P(O)CH2P(O)(OH)2
OPh
IX

SCHEME 1


R = C18H37–
(Me3SiO)3P;
VII
H2O
Me3SiOCH2P(OSiMe3)2;
II
H2O
X
XII
XIV
H2, Pd
H2, Pd
XIII
XV
(COCl)2;
PhOH
XVI

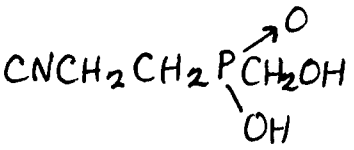
SCHEME 2


NHCOCH3
HOCH2
CH3
CH3
XVII
N=C=N
Me2S→O
O=CH
XVIII
NHCH2CH2NH
CH2
CH2
CH

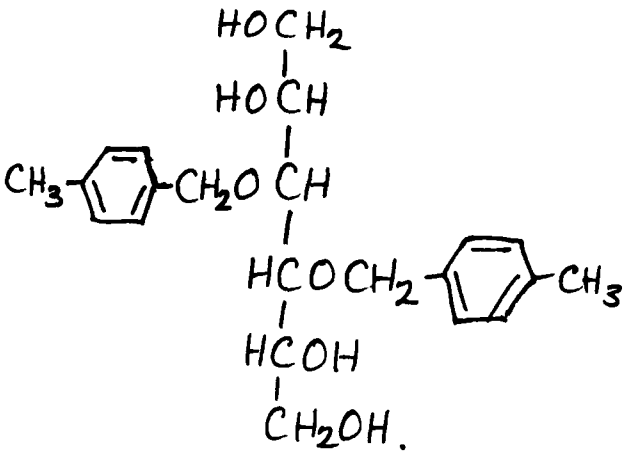
SCHEME 3

COMPOUNDS SUBMITTED

1) 2-Cyanoethyl(hydroxymethyl)phosphinic acid.



2) D-Mannitol 3,4-di(p-methylbenzyl)ether.



SIXTH QUARTERLY REPORT: DAMD-17-76-C-6073

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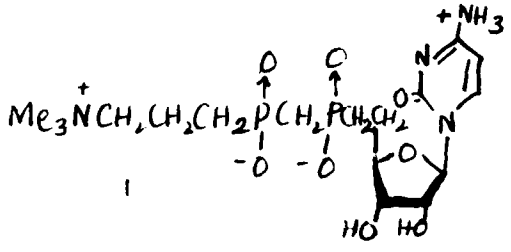
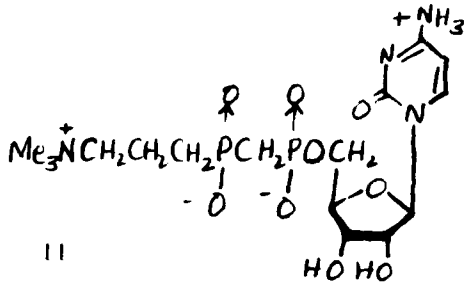
(212) 470-2516

PERIOD: October 1, 1977 to December 31, 1977

During the period covered by this progress report, work continued along the three major fronts which were discussed in the previous progress report. These were: (a) the synthesis of the phosphinate and phosphonate analogs of cytidine diphosphate choline I and II:

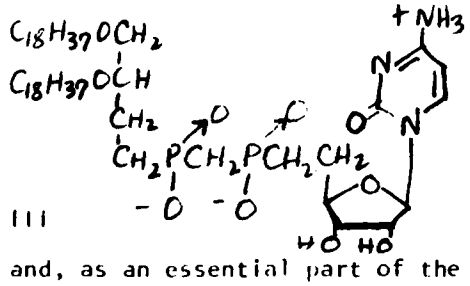


I

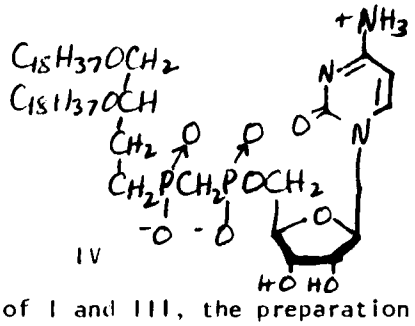


II

; (b) the synthesis of the analogous derivatives of cytidine diphosphate diglyceride III and IV:

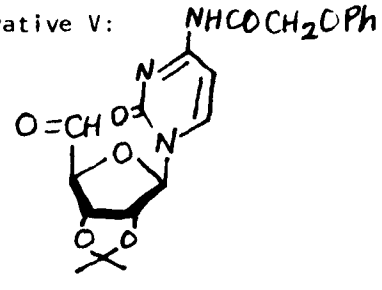


III



IV

; (c) and, as an essential part of the syntheses of I and III, the preparation of the cytidine-5'-aldehyde derivative V:



V

The synthetic route to compounds I - IV have been discussed in detail in the original and second year contract applications and, more recently, in the annual progress report. That portion of the synthesis of I and II which is immediately relevant is given in figure 1. Similarly, that portion of the synthesis of III and IV which is the subject of a portion of the present

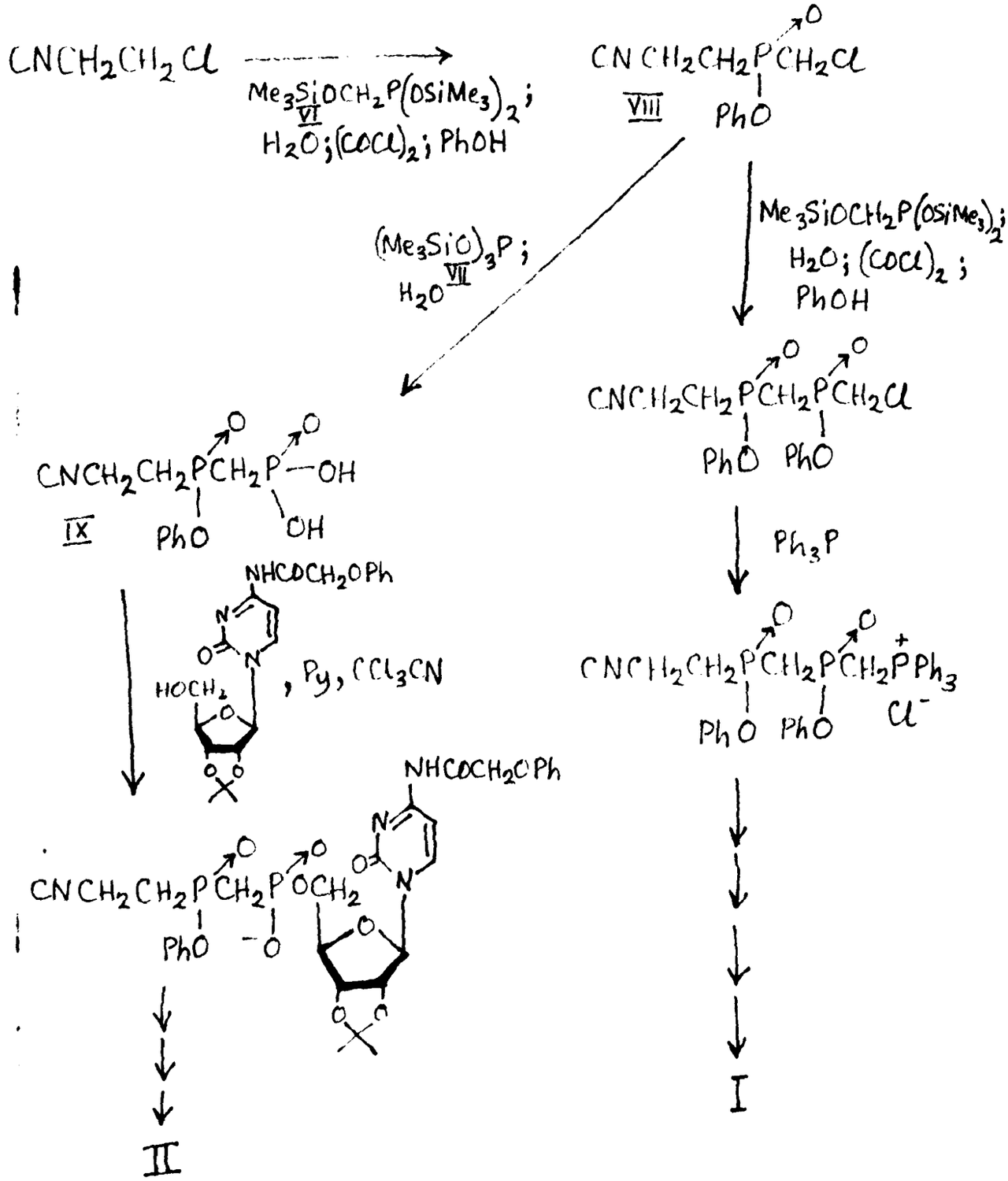

CNCH2CH2Cl
Me3SiOCH2P(OSiMe3)2; VI
H2O; (COCl)2; PhOH
VIII
(Me3SiO)3P; VII
H2O
Me3SiOCH2P(OSiMe3)2;
H2O; (COCl)2;
PhOH
IX
Py, CCl3CN
Ph3P
I
II

Fig. 1

progress report is given in figure 2. Progress made in these two synthetic routes forms the greater part of the present progress report and will be returned to below.

The most exciting development during the period under discussion was the appearance in the literature of a practical method for the synthesis of large amounts of methylenediphosphonous tetrachloride. This potential key intermediate was never available by any practicable synthetic procedure in the past, even though it was well understood that its availability could allow a very facile synthetic route to be substituted for those given in figures 1 and 2, at least with reference to compounds II and IV. Such a synthetic route could avoid many of the difficulties (discussed below) encountered in the older routes and could indeed allow us to concentrate immediately upon the more interesting phosphinate analogs II and IV.

This new route is given in figure 3. It can be seen at once that both phosphorus functions are introduced together from an intermediate containing the two phosphorus moieties in a reactive trivalent form separated by a methylene bridge. This route should allow the synthesis both of the cytidine diphosphate choline and cytidine diphosphate diglyceride analogs with almost equal facility, but utilizing quite different types of starting materials.

The synthesis of methylene diphosphonous tetrachloride itself is quite interesting from a theoretical standpoint, as well as being adaptable in practice to the preparation of quite large quantities of this key intermediate. Aluminum metal (as a coarse powder) is allowed to react for some time with refluxing methylene chloride in the presence of somewhat more than a catalytic amount of methylene bromide (1,2).The reaction which ensues is well controlled and ultimately leads to almost complete dissolution of the metal producing, albeit a large excess of methylene chloride is used, the methylene bis-

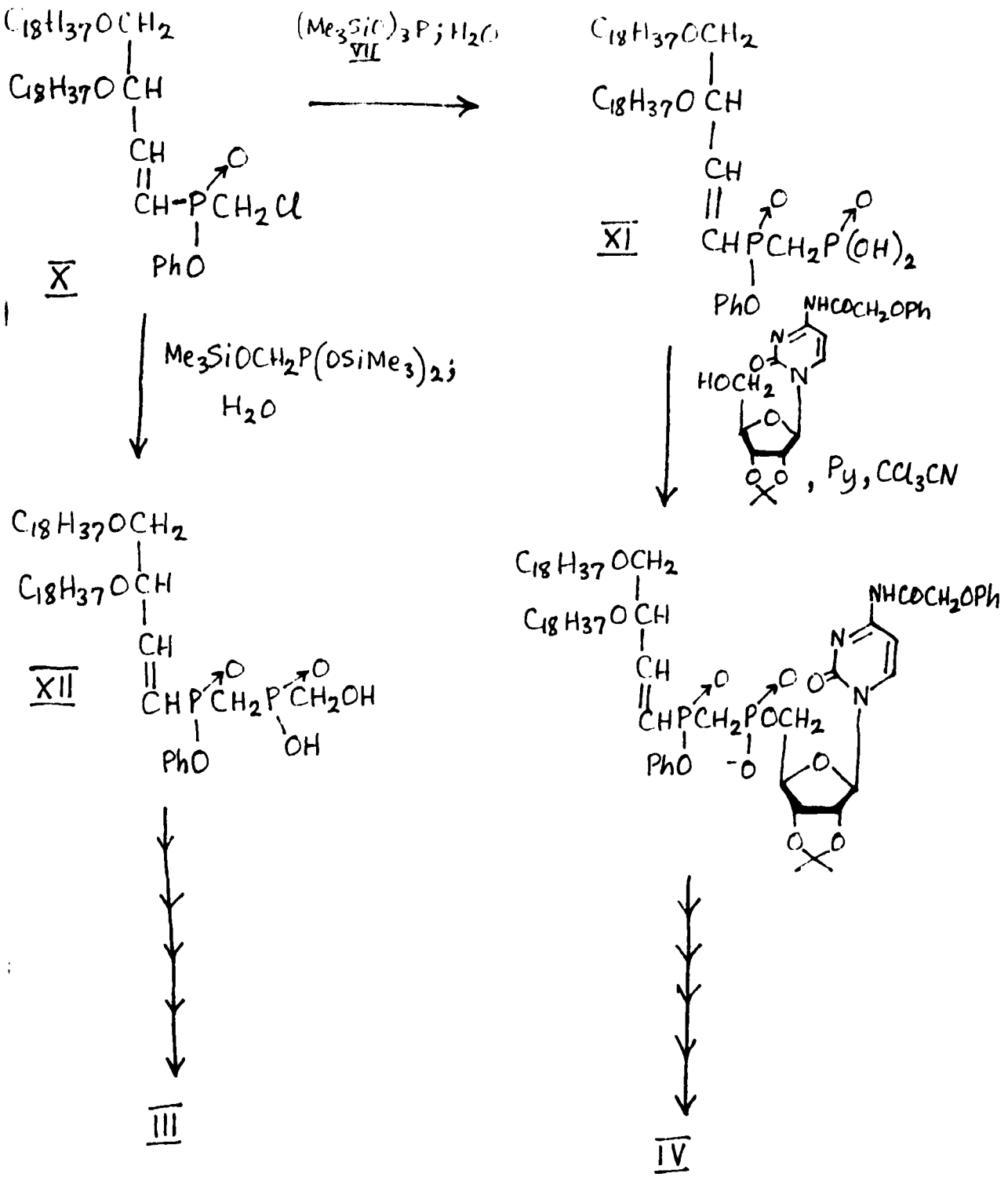

C18H37OCH2
C18H37O CH
CH
CH-P CH2 Cl
PhO
X
(Me3SiO)3P; H2O
VII
Me3SiOCH2P(OSiMe3)2; H2O
C18H37OCH2
C18H37O CH
CH
CHP CH2P(OH)2
PhO
XI
NHCOCH2OPh
HOCH2
Py, CCl3CN
C18H37OCH2
C18H37O CH
CH
CHP CH2P CH2OH
PhO
OH
XII
III
C18H37OCH2
C18H37O CH
CH
CHP CH2P OCH2
PhO
-O
NHCOCH2OPh
IV

Fig. 2

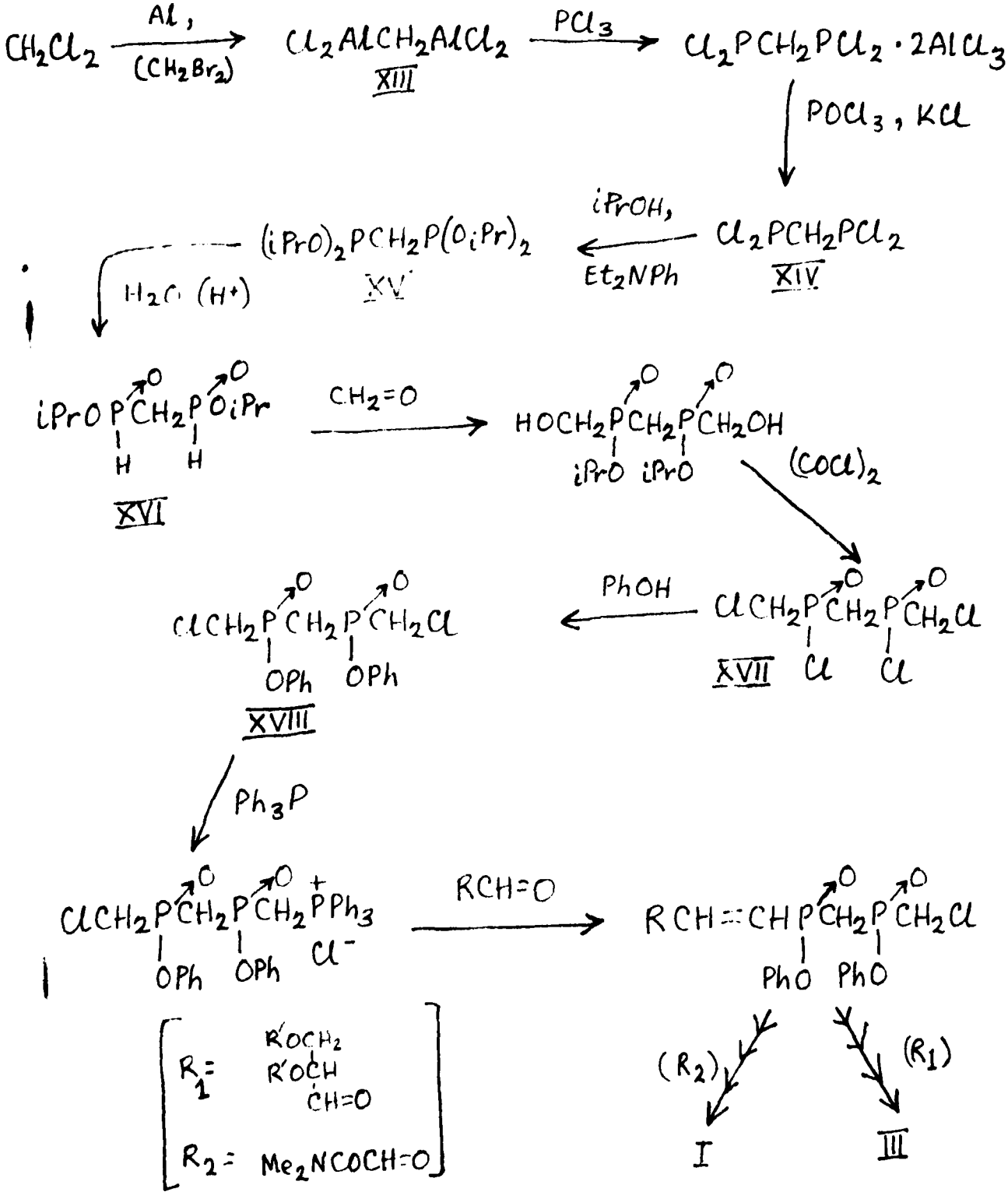

CH2Cl2
Al, (CH2Br2)
Cl2AlCH2AlCl2
XIII
PCl3
Cl2PCH2PCl2 · 2AlCl3
POCl3, KCl
Cl2PCH2PCl2
XIV
iPrOH, Et2NPh
(iPrO)2PCH2P(OiPr)2
XV
H2O (H+)
iPrO P CH2 P OiPr
H H
XVI
CH2=O
HOCH2 P CH2 P CH2OH
iPrO iPrO
(COCl)2
ClCH2 P CH2 P CH2Cl
Cl Cl
XVII
PhOH
ClCH2 P CH2 P CH2Cl
OPh OPh
XVIII
Ph3P
ClCH2 P CH2 P CH2 PPh3 Cl−
OPh OPh
RCH=O
RCH=CH P CH2 P CH2Cl
PhO PhO
R1 = R'OCH2, R'OCH, CH=O
R2 = Me2NCOCH=O
(R2)
(R1)
I
III

Fig. 3

aluminum dichloride, $Cl_2AlCH_2AlCl_2$. Exactly why the bis-aluminum compound is formed is not entirely clear, although the authors of this publication provide certain mechanistic hypotheses. In any event, it is clear that this is in fact the case; it seems reasonable to suppose that the monochloroaluminum dichloride may have a much more reactive chloromethyl moiety than does methylene chloride itself. In the key paper referred to above (3), methylene bis-aluminum dichloride is reacted with phosphorus trichloride in what appears to be simply a variant on a classic organometallic synthetic route, well known in phosphorus chemistry, to produce the desired methylene diphosphonous tetrachloride. It is not surprising that the compound in the presence of the byproduct aluminum chloride is obtained as a complex; this is liberated by addition of phosphorus oxychloride and potassium chloride.

This synthetic route was undertaken in the laboratory toward the end of the present report period and the characteristics of all three reactions studied (formation of the organoaluminum compound, displacement of the chloroaluminum groups by phosphorus trichloride, and decomplexation). The first step appears, at least on its surface, to be very different in kind from an analogous Grignard reaction. It does not appear to be particularly characterized by an induction period or inducible by grinding of the metal, or to be autocatalytic. The reaction begins slowly in the presence of methylene bromide, and continues slowly for at least eighteen hours until the aluminum has all dissolved. In short, the reaction exhibits none of the free radical characteristics often so marked in the reaction of magnesium with organic halides.

Although the organoaluminum compound was always handled under nitrogen for the sake of yield and purity, it very fortunately shows no sign of spontaneous inflammability in air otherwise so characteristic of organoaluminum compounds.

Obviously, the large proportion of chlorine in the molecule confers a sufficient degree of non-inflammability to permit essentially routine handling of the organometallic intermediate.

Reaction with phosphorus trichloride is attended by the liberation of appreciable heat, but ordinary dropwise addition and external cooling serve quite well to moderate the reaction. The same is true of the decomplexation reaction with phosphorus oxychloride which immediately follows.

At this point, the authors call for the addition of potassium chloride. It is not entirely clear what the function of this seemingly inert salt is; possibly it forms the double salt potassium aluminumchloride or simply serves some mechanical function during the agitation of the reaction mixture. In any event, it was found highly advantageous to filter the liquid away from the large amount of suspended inorganic salts under nitrogen pressure at this point, prior to distillation. After removal of solvent, methylene phosphonous tetrachloride was obtained in moderate yield, but on a large enough scale to encourage us to repeat the procedure with modifications to improve the yield. Even if this should not prove possible to any great degree, the starting materials are still cheap enough so that large amounts of the tetrachloride can be economically prepared.

Most of the work during this period concerned, as indicated above, the routes given in figures 1 and 2. In a direct continuation of previous work an attempt was made to develop a good means of purifying phenyl 2-cyanoethyl(chloromethyl) phosphinate, a key intermediate in the proposed route leading to the cytidinediphosphatecholine analogs. Since the intermediate is an essentially undistillable oil, a column chromatographic procedure was investigated. Although this produced a pure product, the yield was only about ten percent of the material applied to the column.

This apparently irreversible adsorption of the chloromethyl compound on silica is strikingly reminiscent of the analogous situation when the chloromethyl lipid intermediate X was similarly purified by dry column chromatography. Although this latter result was obtained some time ago, during the present report period a small scale purification by rapid open column liquid chromatography was achieved with much better results.

A study of the reaction between phenyl 2-cyanoethyl(chloromethyl)phosphinate and tris(trimethylsilyl)phosphite was just undertaken toward the end of the report period. Preliminary indications suggested some difficulty in this reaction. A relatively higher molecular weight phosphorus-containing product could not reproducibly be formed in reasonable yield from this reaction; the data obtained, however, was based only on gas chromatographic experiments.

A possibly analogous synthetic problem in the reaction between the chloromethyl lipid X and either tris(trimethylsilyl)phosphite or the homologous trimethylsilyloxymethyl phosphonite VI under a variety of conditions was always found to produce products which were largely devoid of phenyl proton absorptions in the nmr. It seemed possible that the byproduct of the reaction, trimethylsilyl chloride, was bringing about a de-esterification. This reagent has, of course, well known dealkylating properties (4). Another possibility, of course, was that some sort of intramolecular dearylation was occurring.

In order to test these possibilities with a view toward a practical improvement of this synthetic step, the Arbuzov reaction of X with tris(trimethylsilyl)phosphite, normally performed under a very slow stream of nitrogen, was modified by using a steadier nitrogen flow to remove the volatile trimethylsilyl chloride at a higher rate. After working up the reaction mixture, no phenyl absorptions were found in the product. Moreover, a simpler model compound, bis(p-methylphenyl)-[ ]methylphosphonate, was similarly reacted with tris(trimethylsilyl)phosphite [ ] Arbuzov conditions and the reaction terminated only when no starting

material remained. After removal of excess tris(trimethylsilyl)phosphite, the residue was hydrolyzed and extracted with ether and water. Neither phase, after thorough evaporation and dehydration, showed aromatic absorptions in the nmr. The distillates of the Arbuzov reactions, which should have consisted primarily of trimethylsilyl chloride, did show aromatic absorptions, providing a direct proof of the dephenylation side reaction.

If trimethylsilyl chloride were bringing about the dephenylation, it should be possible to inhibit this undesired step by providing a competitive substrate for trimethylsilyl chloride to act upon at the beginning of the reaction. Repetition of the reaction of the chloromethyl lipid X with tris(trimethylsilyl) phosphite in the presence of an excess of triphenyl phosphite produced results, after work-up of the reaction mixture, which were essentially identical with those obtained in the absence of triphenyl phosphite. It must, therefore, be concluded, at least tentatively, that an intermolecular reaction brought about by trimethylsilyl chloride is an unlikely explanation for the dephenylation observed.

By the conclusion of the period covered by this report, work was under way to react the chloromethyl lipid X with the phosphonite VI and to chlorinate and phenylate the crude product, hoping thereby to restore the phenyl group lost during the Arbuzov reaction in the process of adding the second phenyl group required by the reaction sequence. If, however, the loss of the phenyl group actually produces an inhibited or an abnormal Arbuzov reaction, the problem then would prove more difficult.

When the appropriate ylids (5) are ultimately prepared by whatever route, they will require the availability of a cytidine 5'-aldehyde derivative for reaction to the protected cytidine diphosphate choline or cytidine diphosphate diglyceride. Thus, a separate project was undertaken to prepare the requisite

aldehyde. Cytidine 5'-aldehyde derivatives have never been reported in the literature, which appears to have been confined only to uridine and adenine derivatives. Since the first reports of the preparation of these latter aldehydes appeared some 15 years ago (6), the absence of a report on the synthesis of corresponding cytidine derivatives led us to wonder whether there might not be some particular difficulty in their synthesis which was not obvious. During the last two project periods, only small amounts of N^4-phenoxyacetyl-2',3'-isopropylidenecytidine were available for study of the oxidation reaction, and therefore N^4-acetyl-2',3'-isopropylidenecytidine, of which we had a larger supply, was used in preliminary experiments. The method used was that of Moffatt(7), employing dimethylsulfoxide and dicyclohexycarbodiimide as oxidant. Although chromatographic evidence suggested that the oxidation itself proceeded well, it seemed necessary to isolate the product as the much more stable dianilinoethane adduct for storage purposes. Only impure products were obtained during past project periods with the acetyl compound and this experience was similarly repeated during the present project period with the phenoxyacetyl compound. In fact, good results were not obtained until beyond the present project period when a small but apparently essential modification was made in the Moffatt procedure.

EXPERIMENTAL

Purification of crude L-3,4-dioctadecoxybutyl(phenoxyphosphino)methylphosphonic acid. The crude product obtained from the reaction between X and tris-(trimethylsilyl)phosphite, after hydrolysis with water, was dissolved in chloroform (0.44 g in 5 ml) and applied to a 2X 40 cm column containing 42 g of SilicAR CC-7. The column was eluted with chloroform and chloroform-methanol (95:5 to 85:15). The fractions containing the chromatographically pure phosphorus-containing substance were evaporated. The compound dissolved in tetrachloroethylene shows no phenyl absorptions. If deuterochloroform is used for the nmr examination,

some confusion due to the presence of $CHCl_3$ with aromatic protons (both absorbing around 7.3 ppm) may be found.

Reaction between bis(p-methylphenyl) chloromethylphosphonate and tris(trimethylsilyl)phosphite. The chloromethyl compound (0.2 g) was dissolved in 1 ml of tris(trimethylsilyl)phosphite (10 x excess) and the clear solution was heated under a nitrogen current to 140° overnight. After eighteen hours, the clear reaction mixture was tested for the presence of starting material, of which some was found. The temperature was increased to 150° and the mixture was left overnight again. Thin-layer chromatography now indicated the absence of starting material. Most of the excess phosphite was removed in vacuo and the semi-solid residue hydrolyzed with tetrahydrofuran-water (5:1). After removal of solvent, the mixture was extracted with water and ether. The product should be soluble in the water phase, which was acidic, but the nmr spectrum of the aqueous layer residue, after removal of water and dehydration by thorough evaporation with isopropyl alcohol, does not show any aromatic absorptions. The dried and evaporated ether phase also failed to show these expected peaks.

Purification of phenyl R-3,4-dioctadecoxybut-1-enyl(chloromethyl)phosphinate.

One gram of the crude material in 25 ml of hexane was applied to a column containing 84 g of SilicAR CC-7. The solvent system used for the purification was ethylacetate-hexane, 5 percent to 25 percent. The chromatographically pure product factions were combined and evaporated to give 0.378 g of product. The nmr spectrum in tetrachloroethylene shows the expected phenyl absorption at 7.3 ppm together with other absorptions in the regions expected.

Reaction in the presence of triphenyl phosphite.

The pure chloromethyl compound (71 mg) and 1 ml of tris(trimethylsilyl) phosphite plus 0.16 ml of triphenylphosphite were heated under nitrogen at 125°

for eighteen hours. After this period, some chloromethyl starting material still remained. Additional tris(trimethylsilyl)phosphite (1 ml) was added and the mixture heated at 130 - 140° overnight under a slow nitrogen current. The semi-solid orange reaction mixture which remained after removal of most of the excess silyl phosphite was hydrolyzed with tetrahydrofuran-water (5:1). After evaporation of the solvents and dehydration by re-evaporation with isopropyl alcohol, the residue was precipitated with acetonitrile and filtered. The product obtained in this way was essentially identical to that obtained in the absence of triphenylphosphite.

Methylene bis(aluminum dichloride). Into a 100 ml three-necked flask equipped with a reflux condenser, nitrogen inlet, dropping funnel and magnetic stirrer was placed 2.7 g (0.1 mol) aluminum filings (99.9% pure; Balzers Coating Material, Tridom Chemical Inc., Hauppauge, N.Y.). The aluminum was moistened with 1 ml methylene bromide (dried over 3A molecular sieve). The flask was heated in an oil bath at 35° C and 50 ml methylene chloride (freshly distilled from P_2O_5) was added from the dropping funnel over a period of 30 minutes. Several small crystals of iodine were added to the reaction mixture. The mixture was allowed to reflux overnight, during which time all the aluminum reacted. The light brown reaction mixture was passed through a sintered glass filter under positive nitrogen pressure.

Methylene bis(phosphorus dichloride). The filtrate was further diluted with 50 ml methylene chloride and added dropwise to 13.7 g (0.1 mol) of phosphorus trichloride in a three-necked flask equipped with a magnetic stirrer, reflux condenser and nitrogen inlet. Upon completion of the addition, the solution was refluxed for two hours. Phosphorus oxychloride (15.3 g; 0.1 mol) was added dropwise with stirring, followed by the addition of 7.5 g (0.1 mol) potassium chloride. The mixture was again refluxed for two hours and then

allowed to stand overnight at room temperature. The liquid phase was transferred through a glass wool filter under positive nitrogen pressure to a single-necked flask. The solid residue was washed with another 100 ml of methylene chloride and the washing combined with the original filtrate. Methylene chloride was removed by distillation at atmospheric pressure. Distillation at water pump vacuum (50°) removed most of the unreacted PCl_3 and $POCl_3$. The remaining liquid was distilled under high vacuum (55-60°/1 mm Hg) to yield 2.9 g of the desired product (yield = 27% based on weight of aluminum used).

Purification of phenyl 2-cyanoethyl(chloromethyl)phosphinate. To a solution of crude phenyl 2-cyanoethyl(chloromethyl) phosphinate (4.31g) in 50 ml ethyl acetate was added 20 g of silicic acid (SilicAR CC-7). The mixture was taken to dryness on a rotary evaporator and then kept under high vacuum for about one hour to remove traces of ethyl acetate.

The silicic acid with the adsorbed crude product was added to the top of an already prepared column of SilicAR CC-7 in petroleum ether. The column was eluted with pet. ether (500 ml); pet. ether-ethyl acetate; 90:10 (500 ml); pet. ether-ethylacetate, 75:25 (500 ml) and finally with pet. ether-ethyl acetate, 50-50 (500 ml). This last eluate was collected in 15 ml fractions which were analyzed by GLC on a 6 foot column of OV-17 at 200° C. Those fractions containing pure VIII were combined and concentrated under reduced pressure (bath temperature 35°). This yielded 412 mg of GLC-pure 2-cyanoethyl(chloromethyl) phenylphosphinate. This rather low yield is probably attributable either to irreversible adsorption of the chloromethyl compound to the silicic acid of the column or to decomposition of the chloromethyl compound while on the column. We are currently trying to improve this purification step.

Attempted oxidation of N^4-phenoxyacetyl-2',3'-isopropylidenecytidine. The cytidine derivative (209 mg 0.50 mmol) was dissolved in 1.2 ml of

dimethylsulfoxide previously dried over 3A molecular sieve, under a nitrogen atmosphere. Dicyclohexycarbodiimide (309 mg 1.5mmol) was added in 1.2 ml of anhydrous methylene chloride. The mixture was cooled to 0 - 5° and, with stirring, 20 µl of dichloroacetic acid was added. A precipitate of dicyclohexylurea began to form almost at once. The cold bath was removed and the mixture stirred at room temperature for 90 minutes.

With continued stirring, a solution of 126 mg (1 mmol) of oxalic acid in 1.5 ml of methanol was added. After 30 minutes at room temperature, the mixture was filtered and the filtrate washed thoroughly with cold methanol. Thin-layer chromatography at this point indicated that the oxidation was substantially complete, giving a Schiff-positive product. To the filtrate was added a solution of dianilinoethane (127 mg 0.60mmol) in methylene chloride (1.5 ml). The mixture was allowed to stand for 15 minutes and then evaporated in vacuo at 35 degrees to remove volatile solvents. The residue was kept at room temperature for 30 minutes after addition of a little methanol to effect solution. No appreciable precipitate appeared either at room temperature or on cooling for several hours; a little water was added until precipitation just began and the mixture kept overnight at -5°. The precipitate was filtered and dried in vacuo. It was a mixture of substances, including a transformed cytidine derivative and contained dianilinoethane and dicyclohexylurea. The material was only partly soluble in chloroform and insoluble in hexane. Several crystallizations from chloroform-methanol failed to yield a pure product of the desired structure. The material obtained was still contaminated with appreciable byproducts. Later work indicated that the problem was only in the formation of the dianilinoethane adduct, rather than in the oxidation itself. A modification was then developed which allowed preparation of the pure adduct.

Compound Submitted:

[Phenoxy(chloromethyl)phosphinomethyl] triphenylphosphonium chloride,

$[Ph_3PCH_2P(O)(OPh)CH_2Cl]^+Cl^-$

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(4) R. Rabinowitz. The reaction of phosphonic acid esters with acid chlorides. A very mild hydrolytic route. J. Org. Chem., 28, 2975-2978 (1963).

(5) For this portion of the synthetic route, see Annual Report, Figs. 3b and 4b.

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(7) R.S. Ranganthan, G.H. Jones,and J.G. Moffatt. Novel analogs of nucleoside 3',5'-cyclic phosphates. 1. 5'-Mono- and dimethyl analogs of adenosine 3',5'-cyclic phosphate. J. Org. Chem., 39, 290-298 (1974).

SEVENTH QUARTERLY REPORT: DAMD-17-76-C-6073

CONTRACTOR: Long Island Jewish-Hillside Medical Center

PRINCIPAL INVESTIGATOR: Dr. Arthur F. Rosenthal
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Long Island Jewish-Hillside
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New Hyde Park, N.Y. 11040
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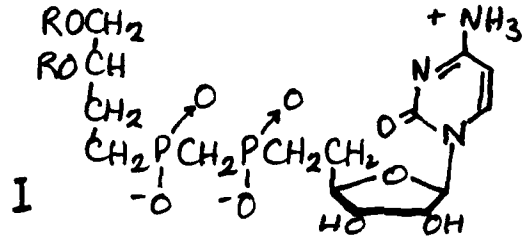
PERIOD: January 1, 1978 to March 31, 1978

During this period the 5' - oxidation of N^4-phenoxyacetyl 2', 3' - isopropylidene cytidine was brought to fruition, making available the 5'-aldehyde as the nucleoside analog portion of the molecule when an appropriate Wittig intermediate becomes available. This should then allow the Wittig reaction to proceed and permit the synthesis of the analogs of types I and II:



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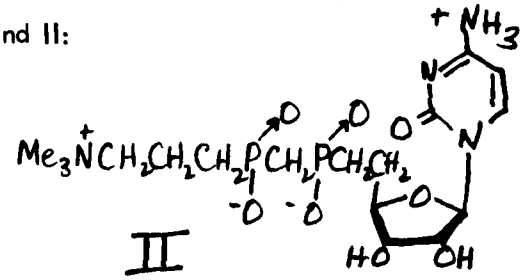


II

To recapitulate briefly, the oxidation to produce uridine and adenine 5'-aldehyde was reported in the literature some time ago (1) but no corresponding reaction of the cytidine derivative was ever published, leading us to wonder whether or not some specific difficulty might have prevented such a reaction from occurring satisfactorily. Indeed, our work during the preceding report periods indicated that, although the oxidation of the cytidine derivative appears to proceed very well, the formation of the aldehyde dianilinoethane derivative according to the procedure published for the uridine and adenine derivatives failed to give a satisfactory cytidine 5' - aldehyde derivative. Experiments with model aldehydes indicated that the catalytic quantity of acetic acid ordinarily employed in the formation of dianilinoethane derivatives but omitted in the published work on the nucleosides could in fact be lowered in concentration to virtually a trace without affecting the adequacy of the catalysis. Thus, it was then found that although omission of acetic acid from the reaction mixture according to the procedure for uridine and adenine derivatives failed to give an adequate product, inclusion of no more than a trace of acetic acid rapidly produced a dianilinoethane derivative in good yield from the reaction mixture. Since the 5' aldehydes readily condense with the 2-hydroxy group of the pyrimidine ring to form cyclo derivatives, the dianilinoethane derivative was essential for certain characterization of the product. However, it would also be possible for synthetic purposes to prepare the aldehyde without derivatization just before use.

In the literature preparation of the free aldehyde of the uridine or adenine derivatives from their dianilinoethane adduct, hydrolysis is accomplished with stirring at room temperature for i 1/2 hrs with a strongly acidic resin. This procedure was studied with our compound, together with a number of alternative possibilities for preparing the free aldehyde from the dianilinoethane adduct without removing the protective isopropylidene or phenoxyacetyl groups. In all cases the results were inferior to simply employing the aldehyde as prepared by oxidation without derivatization;

variable amounts of deprotected products were found in those cases in which the dianilinoethane group was adequately removed. Thus, although the adduct was satisfactorily employed for characterization purposes, it cannot be easily used by any known procedure for synthetic purposes and the 5'-aldehyde must therefore simply be prepared just before use in a synthetic reaction.

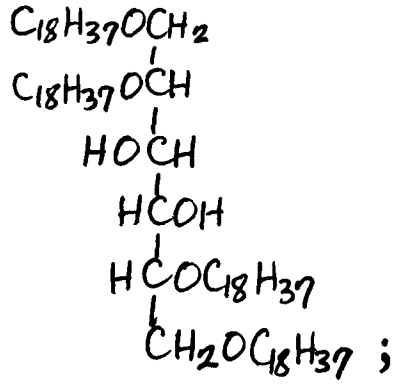
During the last quarterly period efforts were reported which were designed to improve the Arbuzov reaction of phenyl R-3,4- dioctadecoxybut- 1- enyl (chloromethyl) phosphinate with tris-trimethylsilyl phosphite in an effort to prevent the dephenylation which obviously was accompanying the reaction. In addition to removing what might be a valuable protecting group, this side reaction might have also interfered with the primary Arbuzov reaction itself by producing negative phosphorus-oxygen ions. Efforts to surpress the dephenylation by employing a competitive phenyl ester (triphenyl phosphite) in the reaction mixture were unsuccessful, but the question of whether some procedure which would prevent the formation of free negative ions in the reaction mixture could improve the reaction was not explored until the present report. The chloromethyl phosphinate phenyl ester and tris (trimethylsilyl) phosphite were reacted in the presence of bis (trimethylsilyl)-acetamide, a neutral but powerful silanating agent which should react with free oxygen anions as soon as they are formed in the reaction mixture. Even with the inclusion of this reagent, however, no significant improvements in the purity or yield of the reaction product was observed.

Much effort was expended during the present report period into progressing with the new synthetic route based on methylenedisphosphonous tetrachloride, according to the steps shown in figure 1. A large batch of tetrachloride was prepared, with a few simplifications in the synthetic procedure, but maintaining approximately the same yield percentage as before. From the tetrachloride was prepared tetraisopropyl methylenedisphosphonite according to the published procedure (2). The tetrachloride was hydrolyzed, using one equivalent of water and a catalytic quantity of trifluoroacetic acid. When the report period ended further reaction of the diisopropyl dihydrogen methylenediphosphonite with formaldehyde had not yet been undertaken.

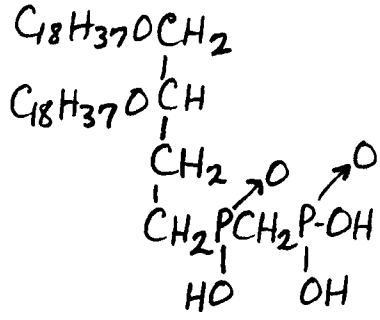
Reaction of the tetraisopropyl ester with an excess of methylene bromide was also studied as a potentially simple alternative to formaldehyde addition and halogenation. The reaction produced and extremely viscous material which was probably polymeric, although it had not been definitively analyzed by the end of the project period. The indications, therefore, are that some form of formaldehyde addition and halogenation would provide the best route to the desired intermediates.

In point of time approximately half of the total project period was actually consumed in the prepar-

ation of larger amounts of intermediates than had previously been reported, and which were required because their supply had become too low to enable the work to proceed adequately without additional preparation. Compounds previously reported but now resynthesized on a larger scale during the present project period were the following:



$Cl_2PCH_2PCl_2$; $(Me_3SiO)_3P$;



EXPERIMENTAL

Oxidation of N^4 - phenoxyacetyl 2', 3'-isopropylidene cytidine. N^4- Phenoxyacetyl 2', 3'-isopropylidene-cytidine (91.45 g, 2.5 m. mol) was dissolved in 6 ml dry dimethylsulfoxide. Dicyclohexylcarbodiimide (1.55 g. 7.5 m. mol) was added in 6 ml of anhydrous methylene chloride. The mixture was cooled to 0°-5° and with stirring 100 ml of dichloroacetic acid was added. A ppt of dicyclohexylurea began to form after ca. 1 min. The mixture was stirred for 90 min in 0-5°.

With stirring a solution of 0.63 g. (5 m. mol) of oxalic acid in 9 ml of methanol was added. After 90 min at -5° the mixture was filtered (at -5°) and the precipitate washed thoroughly with cold methanol.

To the filtrate was added a solution of 0.64 g. dianilinoethane in methylene chloride containing a trace of acetic acid and volatile solvents were removed in vacuo at 35°. Then to the solution a little methanol was added to the first cloudiness; a voluminous precipitate formed rapidly and the mixture was kept overnight at -5°.

It was filtered and the precipitate was dried in vacuo. The crude product weighed 1.52 g; m.p. 178°-183° c, R_f = 0.66 in a n-propanol, -acetone-chloroform, 5:15:80. TLC indicates some UV absorbing impurities. The product was recrystallized from methanol containing a little chloroform at -5°. Finally, 0.87 g of pure product was obtained, m.p. 190°-195°.

Anal:

		%		%
Calculated:	C_{34}	66.98	Found:	66.63
	H_{35}	5.79		5.86
	N_5	11.49		11.47

Deprotection experiments: The hydrolysis of the dianilinoethane adduct of phenoxyacetyl-isopropylidene cytidine would yield the aldehyde if the deprotection was absolutely specific, but if the other protective groups were to be at least partially removed, the fragments would be unfamiliar in terms of the standars we have available. The same would be true if a 5'-2-cyclic condensation were occurring. With the knowledge that in all cases given in the literature either hydrochloric acid deprotection or the presumably milder procedure of stirring at room temperature with a strongly acidic iron exchange resin was necessary to remove the dianilinoethane group, the experiments were done using instead N^4- phenoxyacetyl -2', 3'-isopropylidenecytidine itself. This has the advantage that the fragments produced by unwanted hydrolysis during any deprotection reaction are all availabe to us as chromatographic standards. Thus, providing that our assumption of the conditions necessary for removal of dianilinoethane are correct, this approach should give a valid indication of the likelihood of selective deprotectian.

Phenoxyacetyl-isopropylidenecytidine was subjected to the conditions employed in the literature (1) for removal of the dianilinoethane group; i.e., stirring at room temperature for 90 minutes with Amberlite IR-120, using 1:1 tetrahydrofuran-water as a solvent. Besides the starting material, thin layer chromatography also showed isopropylidenecytidine and even cytidine at the conclusion of the reaction. These extraneous products were formed in even larger amounts when the reaction was allowed to proceed for 12 hrs. Attempts to reduce the removal of the isoproylidene and the phenoxyacetyl groups by lowering the reaction temperature and/or time of reaction were not successful, isopropylidenecytidine and cytidine always appearing at the conclusion of the reaction. When an attempt was made to substitute a weakly acidic resin, no reaction was evident after 90 minutes at room temperature. Such a treatment, however, does not adequately remove the dianilinoethane group.

During the initial oxidation of the protected cytidine to its 5'-aldehyde a quite pure product is

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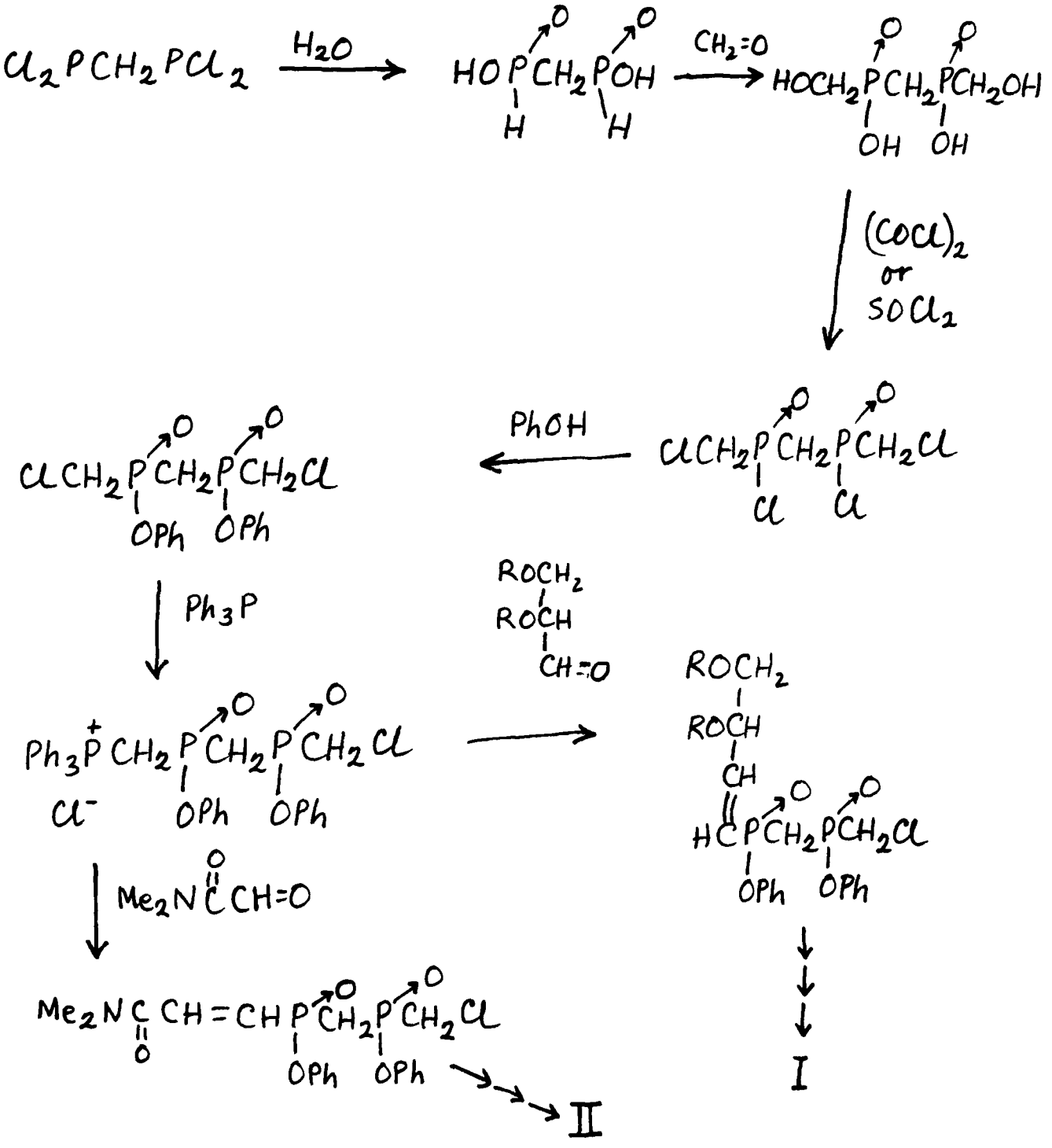
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H2O
HOP(O)(H)CH2P(O)(H)OH
CH2=O
HOCH2P(O)(OH)CH2P(O)(OH)CH2OH
(COCl)2
or
SOCl2
ClCH2P(O)(Cl)CH2P(O)(Cl)CH2Cl
PhOH
ClCH2P(O)(OPh)CH2P(O)(OPh)CH2Cl
Ph3P
Ph3P+CH2P(O)(OPh)CH2P(O)(OPh)CH2Cl Cl-
ROCH2
ROCH
CH=O
ROCH2
ROCH
CH
HCP(O)(OPh)CH2P(O)(OPh)CH2Cl
Me2NC(=O)CH=O
Me2NC(=O)CH=CHP(O)(OPh)CH2P(O)(OPh)CH2Cl
I
II

Fig. 1

formed and it seemed unnecessary at this stage to attempt to work out conditions which would duplicate the formation of this product in the same purity from its dianilinoethane adduct. Thus, we draw the conclusion from this work that cytidine derivatives can be oxidized quite as readily as uridine or adenine derivatives to the 5'-aldehyde, and that this aldehyde should be prepared just before use in a synthetic reaction.

Reaction of Phenyl R-3, 4-dictadecoxybut-1-enyl (chloromethyl) phosphinate with tris (trimethylsilyl) phosphite in the presence of bis (trimethylsilyl) acetamide (BSA).

The chloromethyl compound (30 mg) and 1 ml. of tris (trimethylsilyl) phosphite plus 1 ml of BSA were heated under a nitrogen stream at 140° for 18 hours. After this period, some chloromethyl starting material still remained and the reaction was continued for another 16 hours. Only traces of the chloromethyl starting material remained. After distillation of excess silyl phosphite in vacuo, the residue was hydrolyzed at room temperature in tetrahydrofuran-water and the mixture evaporated as thoroughly as possible. Dehydration was accomplished by repeated re-evaporation with ispropyl alcohol. The product was precipated with acetonitrile at room temperature. Thin layer chromatography of the filtered and dry precipatate showed no improvement of the yield of the desired compound or any simplification of the composition of the product, compared to that obtained in the absence of BSA.

Large-scale preparation of methylene-bis (phosphonous dichloride). All reactions and procedures were carried out under nitrogen. Aluminum (99.9%; 0.2 - 0.7 mm mesh) 405 g; 15 g. atoms was placed in a 3-necked 5 liter flask equipped with a mechanical stirrer, 1 liter dropping funnel and a water-cooled condenser. The aluminum was moistened with 25 ml methylene bromide. As soon as the vigorous reaction started, methylene chloride (distilled from P_2O_5) (5 liters containing as additional 100 ml. of methylene bromide) was slowly added to the aluminum. The reaction flask was heated to reflux temperature and was stirred until all the aluminum had reacted (approximately 36 hours.)

The dark solution was then slowly pressure filtered through glass wool into a 3-necked 12 liter flask (equipped with condenser and N_2 inlet) that had been charged with a solution of freshly distilled phosphorus trichloride (2060 g; 1308 ml; 15 moles) in 1 liter methylene chloride. The rate of addition was adjusted so that the highly exothermic reaction maintained the mixture at the reflux temperature. When all of aluminum alkyl had been added, the mixture was maintained at the reflux temperature for 4 hours.

Then, phosphorus oxychloride (2300 g; 1377 ml; 15 moles) was added slowly from a dropping funnel with vigorous stirring followed by dried potassium chloride (1118 g; 15 moles). The mixture was maintained at reflux temperature until the next day.

Methylene chloride was removed by distillation at atmospheric pressure. Unreacted PCl_3 and $POCl_3$ were removed by distillation at atmospheric pressure and at water pump vacuum. The mixture solidified. The solid mixture was heated with three infrared lamps as well as by the oil bath (at 145°). High vacuum distillation yielded the desired product (55-65°/ 0.8 mm. Hg.) Yield 253 ml (411 g; 25.1%) Desnity=1.625.

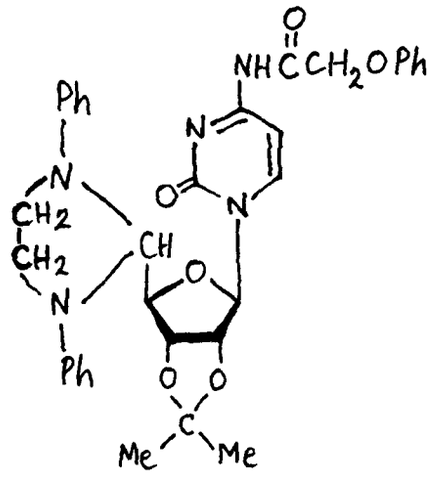
Tetraisopropyl methylenediphosphonite. A solution of 2-propanol (24 g; 0.4 moles) and N,N-diethylaniline (59.6 g; 0.4 moles) in anhydious ether (175 ml) was cooled to -50° under an atmospheric of N_2. To this solution was added dropwise and with stirring a solution of methylene-bis (phosphonous dichloride) (21.8 g; 0.1 mole) in 50 ml ether. The reaction mixture was kept under N_2 throughout. When all the methylene-bis-(phosphorous dichloride) had been added, hexane (100 ml) was added and the stirred mixture was allowed to room temperature and stirred overnight under an atmosphere of N_2. The liquid phase was removed by filtration under positive N_2 pressure. The white precipitate of N, N-diethylaniline hydrochloride was washed with another 100 ml of hexane and the washing combined with the original filtrate. Diethyl ether and hexane were removed by distillation at atmosphere pressure (bath to 100°c). Distillation at 1 mm (70°) bath temperature 100°c) removed a few milliliters of pale yellow liquid (probably unreacted N, N-diethylaniline). The bath temperature was increased to 140° and 17.8 ml (16.7 g) of colorless distillate (98-100 1.5 mm Hg) were collected as the product (54%). The vacuum was increased to 0.11 mm. and a further 4-5 ml of distillate were collected ($b_{0.11}$ 76°).

Diisopropyl dihydrogen methylene diphosphonite. A solution of water (0.9 ml; 0.05 moles) and trifluoroacetic acid (3 drops) in 2-propanol (25 ml) was cooled to -50° under an atmosphere of N_2 to this solution was added dropwise with stirring tetraisopropyl methylenediphosphonite (7.8 g; 0.025 moles). When all of the tetraisopropyl methylenediphosphonite had been added, the solution was allowed to warm to room temperature and stirred for 2 hrs. An additional 0.5 ml water was added and the solution concentrated on the rotary evaporator (bath 35°). An additional 10 ml of 2-propanol were added and the solution was again concentrated on the rotary evaporator. Yield 5.7 gm. (100%). The product was a clear somewhat vicous liquid. In the nmr the $-PCH_2P-$ triplet centered at 8=2.6 ppm (j=25 Hz) and the P-H groups (exchangable) centered at 7.37 ppm of characteristically large (J=567Hz)were clearly evident.

Methylene bis (bromomethylisopropylphosphinate). A solution of methylene-bis-(diisopropylphosphonite) (7.8 g; 0.025 moles) in dibromomethane (25 ml; 6-fold excess) was stirred under a gentle stream of N_2 at 100° in an Arbuzov apparatus for 12 hrs. Through the reflux condenser was circulated water at 65° so that the 2-bromopropane produced in the reaction mixture would not condense. The excess dibromomethane was removed by distillation at atmospheric pressure. This left a very viscous residue which has not yet been analyzed.

COMPOUND SUBMITTED

N^4- Phenoxyacetyl - 2',3'- isopropylidenecytidine
5'- aldehyde, 1,2- dianilinoethane derivative.



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